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EFFECTS OF PHOTOPERIOD ON THE HYPOTHALAMUS AND ON LACTOTROPES IN THE ANTERIOR PITUITARY GLAND OF HOLSTEIN BULL CALVES

By

Steven Andrew Zinn

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ABSTRACT

EFFECTS OF PHOTOPERIOD ON THE HYPOTHALAMUS AND LACTOTROPES IN THE ANTERIOR PITUITARY GLAND OF HOLSTEIN BULL CALVES

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To determine the effects of photoperiod on serum prolactin, activity of tuberoinfundibular dopaminergic (TIDA) and 5-hydroxytryptaminergic (5HT) neurons in the hypothalamus and secretory capacity and number of lactotropes in the anterior pituitary gland, prepubertal Holstein bull calves were utilized. To validate methods the effects of euthanasia with sodium pentobarbital on concentrations of dihydroxyphenylacetic acid (DOPAC) in the median eminence of rats and the effects of elevated serum concentrations of prolactin on accumulation of dihydroxyphenylalanine (DOPA) in the pituitary stalk (PS) of bull calves were determined.

Since euthanasia with sodium pentobarbital did not mask stress-induced changes in activity of TIDA neurons, sodium pentobarbital was an acceptable agent for euthanizing animals in subsequent studies.

Concentrations of dopamine were greatest in the PS in bulls and accumulation of DOPA in the PS was increased after 25 h of haloperidol or after 25 h of infusion of prolactin. Accumulation of DOPA in PS was increased after

9 d of infusion of PRL.

Compared with bull calves exposed to photoperiods of 8 h of light (L):16 h of dark (D) exposure to 16L:8D increased serum concentrations of prolactin 5-fold, secretory capacity and number of lactotropes in the anterior pituitary gland 70 and 16%, respectively and release of PRL into the media from pituitary gland explants 57%. 16L:8D increased accumulation of DOPA in the PS 14%.

In conclusion, 16L:8D-induced increases in serum prolactin are associated with increased number and secretory capacity of the lactotropes. After 4 wk of exposure to 16L:8D the elevated serum concentration may feed back to stimulate accumulation of DOPA.

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LIST OF ABBREVIATIONS

BSA	bovine serum albumin
cAMP	cyclic adenosine 3',5'-monophosphate
D	dark
DA	dopamine
DMEM	Delbecco's modified Eagle's medium
DOPA	dihydroxyphenylalanine
DOPAC	dihydroxyphenylacetic acid
FSH	follicle stimulating hormone
GH	growth hormone
GnRH	gonadotropin-releasing hormone
HEPES	N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid
5HIAA	5-hydroxyindoleacetic acid
5HT	5-hydroxytryptophan
HPLC	high performance liquid chromotography
INS	insulin
IP	inositol phospholipids
iv	intravenous
L	light
LH	luteinizing hormone
MAO	monoamine oxidase
MBH	mediobasal hypothalamus
M199	medium 199
n	number
NE	norepinephrine
NSD 1015	3-hydroxybenzyl-hydrazine
oRBC	ovine red blood cell
pCPA	p-chlorophenylalanine
PRL	prolactin
PS	pituitary stalk
PVN	paraventricular nucleus
SCG	superior cervical ganglion
sc	subcutaneous
SCN	suprachiasmatic nucleus
SE	standard error
T_3	triiodothryonine
T_4	thyroxin
TIDA	tuberoinfundibular dopaminergic
TRH	thyrotropin-releasing hormone
TSH	thyroid hormone stimulating hormone
vs	versus

INTRODUCTION

Photoperiod is a significant cue used by animals in regulation of physiological function in response to changing seasons of the year. Photoperiod can influence reproductive activity and onset of puberty in seasonal breeding species and onset of puberty in non-seasonal breeding species such as cattle and pigs (Diekman and Hoagland, 1983; Petitclerc et al., 1983a). In addition, photoperiod affects many other physiological variables including several production traits important to the livestock industry. For example, exposure to long-day photoperiods of 16 h of light (L) and 8 of dark (D), compared with short-day photoperiods of 8L:16D, increases milk yield 6 to 13% (Peters et al., 1978, 1981; Stanisiewski et al., 1984a), increases mammary development in prepubertal and postpubertal heifers (Petitclerc et al., 1985), increases body growth rate 8 to 17% (Peters et al., 1978; Petitclerc et al. 1983a; Zinn et al., 1986a), increases feed efficiency (Peters et al., 1978; Petitclerc et al., 1983a), increases percentage of protein in the carcass 5 to 11% (Petitclerc et al., 1984; Zinn et al., 1986b) and decreases accretion of fat in the carcass 21% (Zinn et al., 1986b). In addition, exposure to long-day

photoperiods increases concentrations of prolactin (PRL) two- to eight-fold compared with exposure to short-day photoperiods (Bourne and Tucker, 1975; Leining et al., 1979; Stanisiewski et al., 1984b, 1987b).

Although hormonal mediation of photoperiod-induced effects on lactation and growth are yet to be determined, photoperiod-induced changes in PRL have been postulated to play a role (Tucker et al., 1984). Indeed, PRL is an anabolic hormone (McAtee and Trenkle, 1971). For example, infusion of PRL increased nitrogen retention in sheep (Brinklow and Forbes, 1983) and sheep immunized against PRL had reduced growth rates (Ohlson et al., 1981).

Increased production and particularly increased efficiency of production of food producing animals becomes important as the human population increases and available resources for food production decrease. Thus, understanding the mechanism whereby photoperiod affects secretion of PRL may lead to new methods to regulate and enhance efficiency of lactation, mammary development, body growth and carcass composition.

The primary objective of this dissertation was to determine if exposure to photoperiods that alter concentrations of PRL in cattle alter activity of neurons (dopaminergic and 5-hydroxytryptaminergic) in the hypothalamus that regulate secretion of PRL and(or) alter the number and(or) secretory capacity of lactotropes in the pituitary gland. Before the primary objective could be

addressed, validation of the methods was required. Accordingly, additional objectives of this dissertation were: 1) to determine the effects of euthanizing animals with sodium pentobarbital on dopaminergic neurons; 2) to locate potential sites of tuberoinfundibular dopaminergic neurons in cattle and to determine if activity of these neurons can be altered with pharmacological manipulations and 3) to determine if increased activity of these neurons can be maintained over long (9 d) periods of time.

REVIEW OF LITERATURE

A. Effects of Photoperiod on Concentrations of Hormones In Blood

Season of the year influences blood concentrations of several hormones. For example, concentrations of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in blood vary with season in seasonal breeding species (Karsch et al., 1984; Ebling and Lincoln, 1987). Of the anabolic hormones studied, concentrations of PRL are most responsive to changes in season (Tucker, 1982). Season of the year also influences other hormones, such as β-endorphins, and neurotransmitters, such as dopamine (DA) and 5-hydroxytryptamine (5HT; Steger et al., 1985; Ebling and Lincoln, 1987). In this section the effects of season and more specifically the effects of photoperiod on concentrations of hormones and neurotransmitters are described. In addition, the pathways of light signals in regulation of hormone secretion are discussed.

1. Prolactin. Season of the year influences serum concentrations of PRL. For example, elevated serum concentrations of PRL are associated with spring and summer and reduced PRL is associated with fall and winter in

cattle (Koprowski and Tucker, 1973; Kensinger et al., 1979; Peirce et al., 1987), sheep (Ravault, 1976; Munro et al., 1980; Kennaway et al., 1981; Bosc et al., 1982), goats (Buttle, 1974), horses (Johnson, 1986; Thompson et al., 1986), deer (Mirarchi et al., 1978; Bubenik and Schams, 1986) and wild but not domestic pigs (Ravault et al., 1982). In these experiments, animals were exposed to natural uncontrolled seasonal conditions; therefore, the specific factor(s) within season that mediate season-induced changes in PRL could not be determined.

One environmental factor that changes with season, ambient temperature, is positively correlated with seasonally-induced changes in PRL ($r = .61$; Johnson, 1986). In addition, exposure to increased ambient temperature at a constant photoperiod caused a rapid increase in concentrations of PRL, while a decrease in temperature had the opposite effect in cattle (Wettemann and Tucker, 1974; Tucker and Wettemann, 1976) and pigs (Kraeling et al., 1987). Concentrations of PRL were 250% greater in steers exposed to 30°C compared with 10°C (Smith et al., 1977). This temperature-induced increase in PRL was associated with a 3-fold increase in secretion rate and a 36% decline in metabolic clearance rate of the hormone (Smith et al., 1977).

In addition to changes in temperature, length of daily light exposure changes with season, with the longest periods of day light occurring in summer and least in

winter in all but equatorial latitudes. Seasonal changes in photoperiod are constant from year to year whereas seasonal changes in ambient temperature are unpredictably variable. Therefore, photoperiod is a more dependable cue for signalling a change in season than ambient temperature (Hendricks, 1956). Concentrations of PRL and length of day within a season are positively correlated ($r = .80$; Johnson, 1986). To determine the effects of photoperiod on serum concentrations of PRL, independent of temperature, Bourne and Tucker (1975) maintained bull calves at a constant temperature and varied daily light exposure. In one experiment bulls were conditioned to photoperiods of 16L:8D and then over a 12-wk period daily light exposure was gradually reduced to 8L:16D. A second experiment was the reciprocal of the first; exposure began at 8L:16D and gradually increased to 16L:8D. At constant temperatures, decreasing daily light exposure from 16 to 8 h caused an 86% decline in serum concentrations of PRL, whereas increasing light increased PRL 300%. Thus, independent of temperature, changes in daily light exposure altered concentrations of PRL. Similarly, studies in cattle (Leining et al., 1979; Stanisiewski et al., 1984b, 1987b; Crister et al., 1987a), sheep (Forbes et al., 1975, 1979; Fitzgerald et al., 1982; Leshin and Jackson, 1987; Poulton and Robinson, 1987), horses (Johnson, 1987) and deer (Abbott et al., 1984; Bubenik et al., 1987) reported increased serum concentrations of PRL in animals exposed to

daily photoperiods of 16L:8D compared with animals exposed to less than 12 h of light per d. The response of PRL to 16L:8D occurs regardless of the spectral properties of light. For example, PRL was increased when the source of light was from red, blue, Vita-Lite or cool-white fluorescent lights, mercury vapor or high-pressure sodium lamps or incandescent bulbs (Leining et al., 1979; Stanisiewski et al., 1984b).

Although elevated ambient temperature and increasing daily exposure to light increase concentrations of PRL, the speed of response is different. In cattle, temperature-induced changes in PRL occur within minutes whereas the response to photoperiod requires days. In bulls conditioned to 8L:16D, increases in serum concentrations of PRL are not detectable for the first 4 d following an abrupt switch to 16L:8D (Petitclerc et al., 1989) but become significant after approximately 1 wk and reach a maximum 4 to 6 wk after switching to 16L:8D (Leining et al., 1979; Stanisiewski et al., 1984b). The relative sluggishness of photoperiod-induced change in PRL in sheep is similar to that in cattle (Pelletier, 1973; Lincoln et al., 1978).

Once maximal or minimal concentrations of PRL are reached with exposure to 16L:8D or 8L:16D, respectively, no diurnal rhythmicity of concentrations of PRL exists in cattle (Petitclerc et al., 1983b, c). In contrast, sheep

display a marked diurnal increase of PRL at the beginning of the dark period (Lincoln, 1977; Ravault and Ortevant, 1977; Thimonier et al., 1978).

Increasing light exposure to 20 h per d does not increase concentrations of PRL over that obtained with 16 h of light per d (Leining et al., 1979). Exposure to continuous light has produced contradictory results. For example, concentrations of PRL in animals exposed to continuous light were intermediate to concentrations after exposure to 8 and 16 h of light (Kenneway et al., 1983), equal to 8L:16D (Leining et al., 1979) or equal to 16L:8D (Stanisiewski et al., 1987a). The difference in these results may be due to differences in duration of the experiments. Indeed, Stanisiewski et al. (1987a) reported that animals exposed to 24L:0D required an additional 3 wk of exposure for PRL to increase to similar concentrations as bulls exposed to 16L:8D.

Light does not need to be present in a continuous 16 h block of light to increase concentration of PRL. Photoperiod-induced increases in PRL were similar when a short-day photoperiod (> 12 h of light per d) was coupled with a block of light during a photosensitive phase. For example, Ravault and Ortavant (1977) exposed ewes to a photoperiod of 16L:8D or to 7 h of light plus a 1 h block of light 7, 11, 14, 17 or 20 h after the start of the 7 h block of light. When the 1 h block was given 17 h after dawn, concentrations of PRL in these ewes were similar to

PRL in ewes exposed to 16L:8D. The response was reduced when the 1 h block of light was given at other times. Similarly, Petitclerc et al. (1983c) reported that in bulls the response in PRL to a 2 h block of light 14 h but not 20 h after a 6 h block of light was equivalent to 16L:8D. Additional work has shown that photoperiods of 7L:9D:1L:7D or 7L:10D:1L:6D in sheep or 10L:8D:2L:4D in mares increased serum concentrations of PRL as effectively as 16L:8D when compared with PRL in animals exposed to 8L:16D (Thimonier et al., 1978; Schanbacher and Crouse, 1981; Brinklow and Forbes, 1984a; Johnson and Malinowski, 1986). Moreover, Schanbacher et al. (1985) reported that in ewes a 1 min or 15 min block of light 16 h after dawn (7L:9D:1 min or 15 min L) increased serum PRL similar to 16L:8D.

Photoperiod-induced increments in serum concentrations of PRL begin to wane if animals are maintained on 16L:8D for 12 wk (Stanisiewski et al., 1987b). Similarly, concentrations of PRL in sheep maintained on long-day photoperiods for 16 wk begin to decline (Almeida and Lincoln, 1984). Thus, in terms of PRL secretion, cattle and sheep eventually become refractory to photoperiodic stimuli.

2. Gonadotropins. Similar to PRL, photoperiod affects serum concentrations of the gonadotropins, LH and FSH. The direction of photoperiod-induced change in LH and FSH typically corresponds with the breeding season of that

species. That is, when seasonal breeding species are in their reproductively quiescent period serum concentrations of gonadotropins are low and when animals are in their active period concentrations of gonadotropins are elevated. For example, in sheep, a short-day breeding species, serum concentrations of LH and FSH are low in animals exposed to long-day photoperiods and high in ewes exposed to short-day photoperiods (Lincoln et al., 1977). In addition, mares, a long-day breeding species, had greatest concentrations of LH during long-day photoperiods and lowest concentrations during short days (Oxender et al., 1977). These results have been confirmed in sheep (Lincoln, 1979; Lincoln et al., 1982) and in other short-day breeders such as goats (Racey et al., 1975) and white-tailed deer (Mirarchi et al., 1978; Bubenik and Schams, 1986; Bubenik et al., 1987). Similarly, long-day photoperiod-induced increases in concentrations of gonadotropins in long-day breeding species have been confirmed in horses (Clay et al., 1988), golden hamsters (Stegar et al., 1985) and Djungarian hamsters (Yellon and Goldman, 1987). Thus, in contrast to PRL where exposure to long days stimulates secretion in all species studied, exposure to identical photoperiods can have opposite effects on LH and FSH depending on the breeding season of that species.

In non-seasonal breeding species such as swine and cattle, the majority of evidence indicates that photoperiod

does not affect secretion of gonadotropins (Ntunde et al., 1979; Rzepkowski et al., 1982; Diekman and Hoagland, 1983; Peirce et al., 1987; Stanisiewski et al., 1987b, 1988a). In contrast, concentrations of LH may vary with season of the year in ovariectomized cattle. For example, serum concentrations of LH were highest in ovariectomized heifers exposed to photoperiods with increasing light per d than at other times during the year (Day et al., 1986; Crister et al., 1987a; Stumpf et al., 1988).

In rams exposed to lighting regimens of alternating 16 wk periods of 16L:8D and 8L:16D, pulsatile activity of LH and FSH was increased following the switch from long to short days, and reduced following the switch from short to long days (Lincoln, 1979). Similarly, the number of LH pulses were greater during fall and winter than during summer in ovariectomized ewes (5 to 6 pulses/3 h vs 3 pulses/3 h; Karsch et al., 1984). Montgomery et al. (1987) reported similar changes in pulsatile activity of FSH in sheep. The photoperiod-induced increases in gonadotropin pulsatile activity may be mediated by photoperiod-induced changes in secretion of or responsiveness of the pituitary gland to gonadotropin-releasing hormone (GnRH; Hansen, 1985). For example, photoperiod can influence hypothalamic content of GnRH in hamsters (Silverman and Pickard, 1979), white-footed mice (Glass et al., 1988) and sheep (Ebling et al., 1987). In addition, secretion of LH in response to a

constant dose of GnRH (1000 ng, iv) varied with exposure to different photoperiods in rams (Lincoln, 1977) and rusa deer (Van Mourik et al., 1986).

Photoperiod may also influence the responsiveness of the hypothalamic-pituitary axis to negative feedback of gonadal steroids (Karsch et al., 1984). Concentrations of LH and FSH in ovariectomized ewes implanted with estradiol varied with season (Legan et al., 1977; Goodman et al., 1982; Platt et al., 1983). For example, estradiol reduced ovariectomy-induced elevated serum concentrations of LH to undetectable concentrations in ewes in the summer (non-breeding season). However, the ability of estradiol to inhibit LH in ewes in the autumn (breeding season) was reduced, such that serum concentrations of LH were maintained at 10 to 20 ng/ml (Legan et al., 1977). The seasonal effects of estradiol on concentrations of LH are similar when sheep are exposed to controlled photoperiods of 16L:8D or 8L:16D (Bittman et al., 1985). In contrast, implanting ovariectomized cattle with estradiol does not alter season-induced changes in LH secretion (Day et al., 1986; Stumpf et al., 1988).

Similar to PRL, the response of LH and FSH to a stimulatory photoperiod is sluggish. For example, following an abrupt switch from 16L:8D to 8L:16D, serum concentrations of LH and FSH required between 6 and 12 d to increase and 33 to 54 d to reach maximum concentrations (Lincoln and Peet, 1977).

3. Other hormones related to growth. Growth hormone (GH), glucocorticoids, insulin (INS), thyroid hormones, (triiodothyronine [T_3] and thyroxine [T_4]) and β-endorphins may be involved in control of body growth (Gailbraith and Topps, 1981; Schanbacher, 1984; Ebling and Lincoln, 1987). Although not as responsive as PRL, LH and FSH, in some circumstances, these hormones respond to change in season or photoperiod (Leining et al., 1980; Vaughan et al., 1982; Terqui et al., 1984; Denbow et al., 1986; Ebling and Lincoln, 1987). Therefore, the effects of photoperiod on these hormones will be discussed in this section.

In cattle, concentrations of GH are unresponsive to changes in photoperiod. For example, serum concentrations of GH were similar between dairy heifers exposed to 16L:8D or natural short-day winter day lengths (Peters and Tucker, 1978). In addition, photoperiod did not affect several measures of GH in blood; such as average concentrations, secretion rate, metabolic clearance rate, half-life in serum, smoothed baseline, or number or amplitude of pulses (Leining et al., 1980; Petitclerc et al., 1983a; Zinn et al., 1986a, b, 1989). Similarly, photoperiod did not affect serum concentrations of GH in pigs (Kraeling et al., 1983) but in goats that were induced to lactate, concentrations of GH were greater in animals exposed to 15.5L:8.5D compared with 8.5L:15.5D (Terqui et al., 1984).

The influence of photoperiod on GH in sheep is conflicting. For example, Barenton et al., (1987, 1988) reported that concentrations of GH were elevated in 2-year old rams exposed to increasing day length compared with decreasing day length. In contrast, Forbes et al. (1979) and Brown et al. (1979) failed to observed any significant effects of photoperiod on GH in lambs. These differences in photoperiod-induced change in GH may be due to differences in composition of diet and age of the animals used in these studies, both of which influence concentrations of GH (Purchas et al., 1970; Sejrsen et al., 1983).

Similar to sheep, evidence for photoperiod-induced alterations of GH in non-domestic animals are conflicting. Photoperiod does not alter GH in red deer (Brown et al., 1979). However, in white-tailed deer GH is elevated during exposure to short days and reduced during exposure to long days (Bubenik et al., 1975). Similarly, season affects GH in male reindeer and moose (Ryg et al., 1982; Ryg and Jacobsen, 1982). Differences in breed, age and feed availability may account for differences in responses of GH to season in these different species.

Serum concentrations of glucocorticoids were reduced 29 to 58% in 10-wk old bull calves exposed to 15.7, 16 or 20 h of light per d compared with exposure to 8 h of light per d (Leining et al., 1980). In contrast, photoperiod did not affect serum concentrations of glucocorticoids in 4 to 6-

month old or 15-month old heifers (Peters et al., 1980; Zinn et al., 1986b) or lactating cows (Peters et al., 1981). Similarly, exposure to photoperiods of 7L:10D:1L:6D, which mimic long-day photoperiods, reduced serum concentrations of glucocorticoids compared with short-day photoperiods in 3-but not 10-month old lambs (Brinklow and Forbes, 1984a, b). Additional studies indicate that photoperiod did not alter serum concentrations of glucocorticoids in lambs (Kennaway et al., 1981), rams (Lincoln et al., 1982) or white-tailed deer (Bubenik et al., 1975, 1986). However, serum glucocorticoids were increased in lactating sows (Kraeling et al., 1983) and young pigs (Barnett et al., 1981) exposed to 16L:8D compared with 8L:16D.

Differences in glucocorticoid response to photoperiod may be the result of differences in frequency and schedule of blood sampling relative to diurnal changes in glucocorticoids, age or species of animal utilized or time of feeding relative to sampling. All of these variables may influence concentrations of glucocorticoids (Hart et al., 1981; Thun et al., 1981; Lincoln et al., 1982; Brinklow and Forbes, 1984b).

Herbein et al., (1985) and Denbow et al. (1986) reported that serum concentrations of INS were lower in summer than in winter in lactating cows. Similarly, crossbred beef heifers had lower serum concentrations of

INS after exposure to 16L:8D compared with 8L:16D (S.A. Zinn, L.T. Chapin, H.A. Tucker, unpublished observations). In contrast, photoperiod did not influence average concentrations, baseline concentrations or amplitude or number of INS pulses in prepubertal bulls, steers and sheep (Forbes et al., 1979; Tucker et al., 1984; Zinn et al., 1989). These differences may reflect different responses to photoperiod between species (cattle vs sheep) or between sexes (males vs females).

Significant photoperiod-induced changes in thyroid hormone-stimulating hormone (TSH) or thyroid hormones, T_3 and T_4 have been limited to rodents. For example, rats housed in constant darkness had significantly lower serum concentrations of TSH and T_4 than rats housed in constant illumination (Relkin, 1978). Similarly, exposure to short-day photoperiods compared with long-day photoperiods reduces TSH and T_4 in hamsters (Vaughan et al.,1982; Vriend and Wasserman, 1986). However, photoperiod does not influence T_3 or T_4 in cattle (Leining et al., 1980; S.A. Zinn and K. Refsal, unpublished observations) or sheep (Forbes et al., 1979; Lincoln et al., 1982).

In the anterior pituitary gland of rats β-endorphins are derived from proopiomelanocortin in the corticotropes (Weber et al., 1978). Photoperiod can influence blood concentrations of β-endorphins. For example, switching sheep from a photoperiod of 16L:8D to 8L:16D results in a 20-fold increase in plasma concentrations of β-endorphin,

reaching a maximum after 4 to 8 wk of exposure to 8L:16D (Ebling and Lincoln, 1987). The reverse switch in photoperiod led to a decline in β-endorphin (Ebling and Lincoln, 1987). Similarly, greater concentrations of β-endorphin were reported in the mediobasal hypothalamus (MBH) in mice exposed to short versus long days (Glass et al., 1988). However, plasma concentrations of β-endorphin in white-tailed deer were highest in summer and lowest in winter (Bubenik et al., 1988). Although, the role of seasonally-induced changes in β-endorphin is unknown, these changes have been correlated with season-induced changes in gonadotropins in sheep (Ebling and Lincoln, 1987) and antler growth in deer (Bubenik et al., 1988). In addition, β-endorphin has been linked to hyperphagia (Davis et al., 1983) and obesity (Givens et al., 1980). Thus, season-induced changes in β-endorphin may be linked to photoperiod-induced changes in body weight and carcass composition in cattle.

4. Neurotransmitters. Neurotransmitters from the hypothalamus influence synthesis and release of hormones from the anterior pituitary gland (Jacobowitz, 1988). However, only a few studies have focused on photoperiod-induced changes in neurotranmitters. In this section the limited data on photoperiod-induced changes in neurotransmitters, especially DA, 5HT and norepinephrine (NE) will be discussed. GnRH was discussed previously (in

section on gonadotropins) and will not be discussed further.

Anterior pituitary glands from cattle exposed to 8L:16D, compared with cattle given 16L:8D, had greater concentrations of DA but NE and epinephrine were not different (Stanisiewski et al., 1984c). In addition, serum concentrations of PRL were lower in bulls with higher pituitary DA; indicating that photoperiod-induced changes in DA may be a mediator of photoperiod-induced changes in PRL.

In studies using male golden hamsters, Steger et al. (1982, 1984) reported that changing photoperiod can alter hypothalamic concentrations and(or) turnover of DA, 5HT and NE. For example, after 7.5 wk, hamsters switched from long-day (14L:10D) to short-day (5L:19D) photoperiods had reduced hypothalamic concentrations of DA and α-methyltyrosine-induced turnover of DA compared with hamsters maintained on long days. However, after 15 wk concentrations and turnover of DA were not different between hamsters exposed to long and short days. Similarly, hamsters exposed to 5L:19D had greater hypothalamic concentrations of NE but only after 15 wk of exposure to short days (Steger et al., 1982). One d following a shift from short- to long-day photoperiods, hypothalamic 5HT was increased as was the ratio of 5HT to its metabolite 5-hydroxyindoleacetic acid (5HIAA; Steger et al., 1984). In contrast, after 8 wk of exposure to 16L:8D

or 8L:16D, hypothalamic contents of DA, 5HT and NE were similar in the white-footed mouse (Glass et al., 1988).

The conflicting results in these studies may be due to the different species of animals utilized or to differences in time of sampling. Indeed, Benson (1987) reported that photoperiod-induced changes in DA, 5HT and NE are different depending on time of sampling. Compared with exposure to long days, hypothalamic concentrations of DA were greater after 6 wk, lower after 9 wk and not different after 12 wk of exposure to short days in hamsters. Changes in concentrations of neurotransmitters in animals exposed to the same photoperiod for long periods may reflect changes in hormone feed back on neurotransmitter release which may reflect development of refractoriness of the hypothalamus to photoperiodic stimulation.

5. Pathway of light signals in regulation of hormone secretion. Although the exact mechanism whereby photoperiod affects hormone secretion is not completely understood, in most mammals the pathway is considered to begin in the retina and travel via neural connections to the pineal gland (Reiter, 1980). In this section of the review, the pathway that light signals travel to influence hormone secretion is discussed.

The retina is essential for mammals to perceive environmental light cues (Underwood and Gross, 1982). For example, blinding abolishes photoperiod-induced changes in

cattle (Petitclerc et al., 1983b), sheep (Legan and Karsch, 1983), ferrets (Herbert et al., 1978) and hamsters (Reiter, 1969). In contrast, non-mammalian species, such as birds and amphibians, have extra-retinal photoreceptors and therefore do not require the retina to perceive light cues (Okshe, 1965; Van de Kamer, 1965; Follet, 1978). From the photoreceptors in the retina, light signals are transmitted via the retinohypothalamic tract to the suprachiasmatic nucleus (SCN; Moore and Lenn, 1972; Takahashi and Katz, 1982). Similar to the retina, destruction of the retinohypothalamic tract abolishes photoperiod-induced entrainment (Klein and Moore, 1979).

The SCN is a major oscillator of endogenous circadian rhythms in mammals (Stephan and Zucker, 1972; Groos et al., 1983; Turek et al., 1984). In addition, coupling of environmental light:dark cycles and endogenous circadian rhythms occur in the SCN (Klein and Moore, 1979). Destruction of the SCN disrupts seasonal changes in the annual reproductive cycle of sheep (Przekop and Domanski, 1980; Pau and Jackson 1985) and light:dark entrainment of the pineal gland in rats and monkeys (Moore and Klein, 1974; Klein and Moore, 1979; Reppert et al., 1981).

Autoradiographic studies indicate that fibers from the SCN project to the paraventricular nucleus (PVN; Berk and Finkelstein, 1981). The PVN may act as a relay station from the SCN to the pineal gland (Klein et al., 1983). For example, complete destruction of the PVN blocks

photoperiod-induced changes in secretion of melatonin and gonadal function (Pickard and Turek, 1983; Lehman et al., 1984; Nunez et al., 1985).

Neurons pass from the PVN through the medial forebrain bundle and reticular formation to the intermediolateral cell column in the spinal cord (Moore and Klein, 1974; Klein and Moore, 1979). These projections innervate preganglionic cells which in turn innervate the superior cervical ganglion (SCG; Binkley, 1983). Destruction of the SCG inhibits photoperiod-induced changes of PRL in sheep (Lincoln, 1979; Lincoln et al., 1982) and goats (Buttle, 1977; Maeda et al., 1986).

Postganglionic noradrenergic cells in the SCG project to the pineal gland via the inferior carotid nerve and the nervii coronarii (Reiter, 1982; Klein et al., 1983). Postganglionic fibers terminate with the perivascular spaces near the pinealocytes within the pineal gland (Reiter, 1982). NE, released from these nerve terminals, in response to dark binds to β-adrenergic receptors and initiates the synthesis and release of melatonin from pinealocytes (Wurtman and Axelrod, 1966; Binkley, 1983).

Secretions from the pineal gland, especially melatonin, may act as a hormonal signal an animal uses to determine length of day. For example, secretion of melatonin from the pineal gland is lowest during periods of light and greatest during periods of dark (Rollag and Niswender,

1976; Lincoln et al., 1982). Thus, daily duration of the nocturnal rise of melatonin signals the length of darkness to an animal (Bittman and Karsch, 1984). Melatonin does not appear to act directly on the anterior pituitary gland (Bartke et al., 1978), but may act by modulating release of hypothalamic neurotransmitters and(or) neurohormones, which in turn affect release of hormones from the anterior pituitary gland (Reiter, 1980; Karsch et al., 1984).

Pinealectomy suppresses photoperiod-induced changes in sheep (Brown and Forbes, 1980; Brinklow and Forbes, 1984b; Karsch et al., 1984) and hamsters (Reiter and Hestor, 1966; Reiter, 1980). In addition, infusion of melatonin for 16 or 8 h in pinealectomized, ovariectomized estradiol-treated ewes caused changes in LH secretion analagous to those in ewes exposed to short or long days, respectively (Bittman and Karsch, 1984). Similarly, pattern of concentrations of melatonin in serum may mediate photoperiod-induced effects on PRL concentrations (Kennaway et al., 1982, Symons et al., 1983). In contrast, pattern of concentrations of melatonin in serum does not appear to affect secretion of PRL in cattle (Crister et al., 1987b; Stanisiewski et al., 1988a, b). That is, regardless of pattern of melatonin in serum, long-day photoperiods continue to stimulate concentrations of PRL in cattle. Although patterns of concentrations of melatonin in serum may mediate other photoperiod-induced changes in cattle (e.g., fat accretion; Zinn et al., 1988) they do not mediate photoperiod-induced

changes in PRL. Therefore, alternative pathways (in addition to the retino-pineal axis) for perception of light signals must exist. Indeed, lesion of the PVN destroys photoperiod-induced gonadal function but does not affect photoperiod-induced changes in the circadian rhythm of activity in hamsters (Pickard and Turek, 1983; Nunez et al., 1985) further supporting the concept that alternative pathways for photoperiod-induced changes in physiology exist.

6. Summary. The hormone that responds most consistently to changes in photoperiod in seasonal and non-seasonal breeding species is PRL. Concentrations of GH, INS and glucocorticoids are altered with changes in photoperiod in certain species but changes in concentrations in these hormones are not consistent. However, the effects of photoperiod on these hormones are not as consistent as the effects on PRL. Gonadotropins respond to changes in daily photoperiod but are more correlated with changes in reproductive status in seasonal breeding species and photoperiod does not influence gonadotropins in non-seasonal breeding species. PRL is an anabolic hormone and therefore, may be responsible for photoperiod-induced changes in growth, development and lactation in cattle.

The retina is essential for perception of photic signals in mammals. Light signals may be transformed to

endocrine signals via the daily pattern of melatonin secretion. The daily pattern of melatonin may mediate photoperiod-induced changes in gonadotropins and PRL in seasonal breeding species, but not in cattle. Therefore the mechanism whereby photoperiod influences secretion of PRL is unknown.

Neurotransmitters such as DA and 5HT, affect secretion of PRL (Jacobowitz, 1988) and photoperiod can influence activity of DA and 5HT neurons. Photoperiod-induced changes in DA and 5HT, therefore, may mediate photoperiod-induced changes in serum concentrations of PRL.

B. Dopaminergic Control of Prolactin Secretion

Prolactin is secreted from the lactotropes of the anterior pituitary gland. The prevailing hypothalamic control of secretion of PRL is inhibition (Leong et al., 1983). The majority of the tonic inhibition of PRL by hypothalamic factors has been attributed to DA (Clemens and Shaar, 1980). Therefore, in this section of the review, dopaminergic control of secretion of PRL from the lactotropes of the anterior pituitary gland will be discussed.

1. Inhibitory control of prolactin. Transplantation of the anterior pituitary gland to under the kidney capsule (and thus away from direct hypothalamic influence) results

in increased serum concentrations of PRL (Everett, 1954). Serum concentrations of PRL increase following: transection of the pituitary stalk (Chen et al., 1970; Bishop et al., 1971; Woolf et al., 1974), bilateral destruction of the median eminence (Chen et al., 1970) and destruction of the MBH (Arimura et al., 1972; Butler et al., 1975). In addition, acid extracts of hypothalamic tissue inhibited secretion of PRL in vitro (Talwalker et al., 1963). Furthermore, extracts from hypothalamic but not cerebrocortical tissue decreased serum PRL when infused into portal vessels leading to the pituitary gland (Kamberi et al., 1971a), indicating that tonic inhibition of secretion of PRL from the anterior pituitary gland is direct and specific to the hypothalamus.

Infusion of DA directly into hypophyseal portal vessels inhibits secretion of PRL (Takahara et al., 1974a). In addition, antagonists of DA, such as haloperidol, increase secretion of PRL whereas DA agonists, such as ergot alkaloids, inhibit PRL (Clemens and Shaar, 1980; Seeman, 1981). In vitro, DA suppresses secretion of PRL from explants (Takahara et al., 1974b) and dispersed cells (Padmanabhan et al., 1979) of the anterior pituitary gland. The influence of DA on PRL is dose dependent (Padmanabhan et al., 1979; Yeo et al., 1979) and is reversible (Yeo et al., 1979; Thorner et al., 1980). In addition, pretreatment or cotreatment of pituitary cells with DA antagonists prevents DA-induced suppression of PRL

(MacLeod, 1976; Caron et al., 1978; Denef and Follebouckt, 1978; Rick et al., 1979).

Concentrations of DA within hypophyseal portal vessels are greater than in the general circulation (Ben-Jonathan et al., 1977; Gibbs and Neill, 1978) and are altered with physiological state and pharmacological manipulations (Ben Jonathan et al., 1977; de Greef and Neill, 1979). In addition, these concentrations of DA are sufficient to inhibit secretion of PRL in rats (Gibbs and Neill, 1978) and from bovine pituitary cells in vitro (Shaar and Clemens, 1974; Padmanabhan et al., 1979).

Receptors for DA are present on anterior pituitary cells (Brown et al., 1976; Calabro and MacLeod, 1978; Cronin et al., 1978) and in particular on the plasma membrane of lactotropes (Goldsmith et al., 1979).

D_2 (Seeman, 1981) but not D_1 (Cocchi et al., 1987) DA receptors are present on lactotropes. D_2 DA receptors are negatively linked to the adenyl cyclase system (Ben-Jonathan, 1985). The number of DA binding sites on the anterior pituitary gland vary with changes in serum concentrations of PRL. For example, the number of DA binding sites increases coincident with the surge of PRL during proestrus in the rat estrous cycle (Heiman and Ben-Jonathan, 1982). In addition, neonatal rats treated with the neurotoxin monosodium glutamate which selectively destroys retinal and arcuate nuclear neuronal perikarya,

had 3-fold greater concentrations of PRL and 60% more DA binding sites than controls without change in the affinity of receptors for DA (Ben-Jonathan, 1985). Thus, increasing concentrations of PRL result in up-regulation of DA receptors. Conversely, following treatment with bromocriptine, a dopamine agonist, concentrations of PRL are reduced as are the number of DA binding sites (Di Paolo and Falardeau, 1984).

In summary, secretion of PRL from lactotropes in the anterior pituitary gland is tonically inhibited and hypothalamic DA, acting through D_2 receptors directly on the lactotrope, is the primary prolactin inhibiting factor.

2. Intracellular mediators of dopaminergic action in the lactotrope. The events following DA binding to D_2 receptors on the lactotrope to inhibit PRL secretion is not completely understood. Typically extracellular factors, such as DA, that bind to receptors on the plasma membrane of a target cell, are coupled to an intracellular second messenger system to elicit a specific cellular response. Several compounds, including cyclic adenosine monophosphate (cAMP), calcium and inosital phospholipids (IP), may act as intracellular mediators in lactotropes. DA may influence calcium, cAMP and(or) IP individually or in combination to inhibit secretion of PRL (Enjalbert et al., 1988) and may involve more than a single mechanism. Thus in this section of the review the influence of DA on the intracellular

second messengers, cAMP, calcium and IP and their possible interactions will be discussed.

DA binding to D_2 receptors on lactotropes is negatively associated with adenyl cyclase (Enjalbert and Bockaert, 1983; McDonald et al., 1984), a membrane bound enzyme that converts adenosine triphosphate to cAMP (Stryer, 1975). DA in nanomolar concentrations inhibits accumulation of cAMP in dispersed pituitary cells within 1 to 5 min (Swennen and Denef, 1982; Bockaert et al., 1988). Pretreatment with Bordetta Pertussis toxin, which blocks receptor-mediated inhibition of adenyl cyclase, blocks DA-induced reduction in cAMP and PRL (Bockaert et al., 1988). In addition, cholera toxin-induced and thyrotropin-releasing hormone (TRH)-induced increases in cAMP and PRL are inhibited following treatment with DA or DA agonists (Barnes et al., 1978; Gianattasio et al., 1981). Collectively, these data support a role for suppression of cAMP as a mechanism of DA inhibition of secretion of PRL.

Changes in intracellular concentrations of calcium can influence secretion of PRL. For example, exposing cells from anterior pituitary gland to increasing concentrations of calcium increased secretion of PRL (Thorner and MacLeod, 1980). Similarly, treatment with a calcium channel agonist, maitotoxin, increased secretion of PRL (Schettini et al., 1986). Conversely, removal of calcium from the media of pituitary cells cultured in vitro or blocking calcium channels on the lactotropes in vivo, thereby

preventing calcium influx, decreased secretion of PRL (MacLeod and Fontham, 1970; Thorner and MacLeod, 1980). Therefore, DA may inhibit secretion of PRL by reducing intracellular concentrations of calcium. Indeed, DA reduces intracellular calcium in primary cultures of lactotropes (Shrey et al., 1986; Malgaroli et al., 1987a, b; Login et al., 1988). Calcium may directly stimulate secretion of PRL or it may act indirectly. For example, treatment of dispersed pituitary cells with a calcium channel activator, increased calcium influx and increased secretion of PRL, and also increased intracellular concentrations of cAMP (Schettini et al., 1986). Treatment with DA reduced the elevated cAMP as well as the increased PRL (Schettini et al., 1986). Thus, DA may inhibit secretion of PRL by reducing calcium-stimulated production of cAMP.

Hydrolysis of IP in cell membranes is an intracellular second messenger in a number of cell systems (Joseph et al., 1984) including the anterior pituitary gland (Bonetti et al., 1987). IP, in turn, may stimulate release of stored intracellular calcium pools which increases secretion of hormone (Hawthorne, 1983). Increased intracellular IP is associated with TRH- and angiotension II (AII)-stimulated PRL release from anterior pituitary cells (Enjalbert et al., 1986; Law et al., 1988a). In addition, age-related differences in incorporation of ^{32}P

into IP paralleled age-related differences in secretion of PRL (Bonetti et al., 1987). Moreover, inclusion of a IP, hexabisphosphate, in perfusion media increases secretion of PRL from anterior pituitary cells (Law et al., 1988b). Thus, increased intracellular production of IP is associated with increased secretion of PRL. DA may inhibit secretion of prolactin by tonically inhibiting synthesis of IP. Indeed, DA or DA agonists inhibit synthesis of IP in vivo (Bonetti et al., 1987) and in vitro (Bockaert et al., 1988).

Malgaroli et al., (1987a) reported that TRH causes a biphasic increase in secretion of PRL. The initial peak is due predominantly to redistribution of intracellular stores of calcium and a secondary phase is due to influx of extracellular calcium (Valler et al., 1988). Increased PI is associated with an influx of extracellular calcium (Valler et al., 1988). DA does not inhibit release of calcium from intracellular stores associated with TRH-induced rapid accumulation of IP (Valler et al., 1988). However, DA may suppress increased synthesis of IP associated with a secondary influx of extracellular calcium (Valler et al., 1988) and thereby inhibit secretion of PRL associated with this second phase of TRH-induced release.

In summary, several intracellular mechanisms may be involved in dopaminergic inhibition of PRL secretion including mechanisms that regulate intracellular concentrations of calcium, cAMP and IP. The extent of DA

inhibition of PRL may depend on the individual and(or) interactive negative influence DA exerts on calcium, cAMP and IP.

3. Tuberoinfundibular dopaminergic neurons. DA is the primary inhibitor of secretion of PRL from the anterior pituitary gland. The DA primarily responsible for inhibition of PRL is secreted from tuberoinfundibular dopaminergic (TIDA) neurons (Ben-Jonathan, 1985). There is no loss of DA in the median eminence following hypothalamic deafferentiation (Jonsson et al., 1972; Gudelsky et al., 1978) indicating that perikarya and axons of TIDA lie completely within the MBH of the rat (Moore and Johnston, 1982). The TIDA neurons originate in the arcuate and periventricular nuclei within the MBH (Ben-Jonathan, 1985; Albenese et al., 1986). Axons of TIDA neurons are short and project to the external layer of the median eminence (Moore, 1987) and terminate near the perivascular spaces of the capillary plexus of the hypophysial portal system (Ajika and Hokfelt, 1973). Electrical stimulation of the arcuate nucleus increases turnover and synthesis of DA in TIDA neurons and reduces secretion of PRL (Gunnet et al., 1987; Lookingland et al., 1987a).

Tyrosine, the precursor of DA, is taken up by dopaminergic neurons and hydroxylated to L-dihydroxy-phenylalanine (DOPA) by the enzyme tyrosine hydroxylase (Fernstrom, 1983). Tyrosine hydroxylase is the rate-

limiting enzyme in the synthesis of DA (Spector et al., 1967). The enzyme, aromatic-L-amino acid decarboxylase, rapidly decarboxylates DOPA to DA (Moore, 1987), such that intraneuronal concentrations of DOPA are essentially zero (Demarest and Moore, 1980). DA then feeds back to inhibit activity of tyrosine hydroxylase (Spector et al., 1967).

Newly synthesized DA in TIDA neurons can be released into the perivascular spaces of the primary plexus of the hypophyseal portal system and transported via the blood to the anterior pituitary gland to exert its influence on PRL (Moore et al., 1980). In addition, newly synthesized DA can be scavanged by intraneuronal monoamine oxidase (MAO) to the predominant metabolite of DA, dihydroxyphenylacetic acid (DOPAC; Lookingland et al., 1987b). DA can also be stored in synaptic vesicles (Moore, 1987).

Although synthesis and release of DA in all dopaminergic neurons may be similar, there are several unique characteristics of TIDA neurons. For example, unlike other dopaminergic neurons, TIDA neurons do not form synapses but release DA directly into the portal blood (Moore, 1987). Intraneuronal concentrations of DOPAC are lower in TIDA than nigrostriatal DA neurons (Umezu and Moore, 1979), which is most likely due to the reduced affinity for DA of the DA-reuptake system in TIDA neurons (Demarest and Moore, 1979; Annunziato et al., 1980). That is, in TIDA neurons less DA is reincorporated into the neuronal terminals and therefore less DA is available for

metabolism to DOPAC. In addition, TIDA activity is not regulated directly by DA-receptor mediated mechanisms (Demarest et al., 1985b). That is, DA agonists or antagonists do not act directly on TIDA neurons to elicit change in neuronal activity (Gudelsky and Moore, 1976, 1977). For example, injection of haloperidol, a DA antagonist, into rats stimulates TIDA neuronal activity 12 or 24 h but not 2 or 8 h after injection (Demarest and Moore, 1980; Gudelsky and Porter, 1980). Conversely, TIDA activity was decreased 26 h but not 2 h after the start of injections of bromocriptine, a DA agonist (Demarest and Moore, 1981). Effects of haloperidol and bromocriptine on TIDA activity were eliminated in hypophysectomized animals (Demarest and Moore, 1980, 1981) indicating that actions of DA agonists and antagonists on TIDA neurons are mediated through their actions on the pituitary gland.

Since DA antagonists and agonists stimulate and inhibit secretion of PRL from the anterior pituitary gland, respectively, PRL is a likely choice as the mediator of these drugs on TIDA activity (Moore, 1987). Indeed in rats, systemic (Hokfelt and Fuxe, 1972; Gudelsky et al., 1976) and intracerebroventricular (Annunziato and Moore, 1978; Johnston et al., 1980) injections of PRL selectively increased TIDA activity. However, PRL must be elevated for at least 12 h to increase TIDA neuronal activity (Demarest et al., 1985b).

Although the exact mechanism whereby PRL stimulates TIDA activity is unknown, it appears that protein synthesis is involved, since treatment wtih cycloheximide, an inhibitor of protein synthesis blocks the effect of PRL (Johnston et al., 1980). Therefore, DA from TIDA neurons tonically inhibits secretion of PRL and serum concentrations of PRL, in turn, regulates activity of TIDA neurons (Hokfelt and Fuxe, 1972; Gudelsky et al., 1976; Annunziato and Moore, 1978). That is, increased concentrations of PRL feed back to increase TIDA activity and subsequent DA release. Then, reduced secretion of PRL results in decreased TIDA activity and decreased DA release (Demarest et al., 1985b).

The feedback system described above can be interrupted during various physiological states. For example in rats, suckling-induced and restraint stress-induced increases in PRL are associated with decreased TIDA neuronal activity and DA release (Selmonoff and Wise, 1981; Demarest et al., 1985a). In addition, estradiol treatment increased secretion of PRL but reduced TIDA activity by disrupting PRL feedback on the neurons (Demarest et al., 1984). Thus, in certain physiological states TIDA neuronal activity can be inhibited despite high serum concentrations of PRL.

4. Summary. Secretion of PRL is tonically inhibited. DA released from TIDA neurons in the MBH into the hypophyseal portal blood, is primarily responsible for the tonic inhibition of PRL secretion. DA binds to D_2

receptors on the plasma membrane of the lactotrope and by post-receptor mechanisms involving cAMP, calcium and(or) IP, inhibits secretion of PRL. Serum concentrations of PRL feed back to regulate TIDA neuronal activity. However, the feedback system can be interrupted with changes in physiological state. Therefore, it seems reasonable to hypothesize that photoperiod may stimulate secretion of PRL by interfering with the PRL-TIDA feedback mechanism. Indeed, as mentioned in a previous section, photoperiod can alter concentrations of DA in pituitaries of bulls (Stanisiewski et al., 1984c) and concentrations of DA in median eminence of hamsters (Steger et al., 1982, 1984, 1985).

C. 5-Hydroxytryptaminergic regulation of prolactin secretion

Many neuropeptides and neurotransmitters, including 5HT, stimulate secretion of PRL (Jacobowitz, 1988). The 5HT neurons have been localized in several areas within the hypothalamus, including the SCN (Kent and Sladek, 1978; Card and Moore, 1984). The SCN is a part of the neural pathway whereby light signals are transmitted from the retina to the hypothalamus and is implicated in circadian rhythmicity (reviewed in the section on the pathway of light signals in regulation of hormone secretion) including

the circadian release of PRL in rats (Bethea and Neill, 1979; Dunn et al., 1980). Thus, it seems reasonable to speculate that changes in 5HT may be involved in photoperiodic regulation of secretion of PRL. Therefore, in this section of the review 5HT influence on secretion of PRL will be discussed.

1. 5-Hydroxytryptaminergic neurons. The essential amino acid tryptophan is the precursor of 5HT. Tryptophan is transported into 5-hydroxytryptaminergic neurons and converted to 5-hydroxytryptophan (5HTP) by the enzyme tryptophan hydroxylase (Lovenberg et al., 1968). Aromatic-L-amino acid decarboxylase then rapidly converts 5HTP to 5HT (Lovenberg et al., 1962), such that intraneuronal concentrations of 5HTP are essentially zero (Fernstrom, 1983). The resulting 5HT is either released, translocated to synaptic vessicles or converted to its predominant metabolite, 5HIAA, in a two-step process (Baumgarten and Schlossberger, 1984). First, 5HT is converted to 5-hydroxyindolealdehyde acid by MAO and then to 5HIAA by an aldehyde dehydrogenase (Fernstrom, 1983).

Tryptophan hydroxylase is not saturated at physiological concentrations of tryptophan in the brain, and therefore substrate (i.e., tryptophan) availability may be an important factor in control of tryptophan hydroxylase and subsequent synthesis of 5HT (Leathwood, 1987). For example, increasing or decreasing concentrations of tryptophan in the brain increases or decreases rate of 5HT

synthesis and accumulation of 5HIAA, respectively (Fernstrom and Wurtman, 1971; Carlsson and Linquist, 1973; Biggio et al., 1974; Arimanana et al., 1984). In addition, regulation of 5HT synthesis by end-product inhibition of tryptophan hydroxylase has been reported when concentrations of 5HT are increased two to three-fold following treatment with MAO inhibitors (Macon et al., 1971),

Therefore, tryptophan hydroxylase, and thus synthesis of 5HT, may be regulated by several different mechanisms including end-product inhibition and(or) substrate availability.

2. Stimulatory control of prolactin. Infusion of 5HT stimulates secretion of PRL in vivo in a dose-dependent manner (Kamberi et al., 1971a; Lawson and Gala, 1975, 1978; Lawson et al., 1980; Wehrenberg et al., 1980; Thomas et al., 1987). Similarly, treatment with 5HTP, the immediate precursor of 5HT, stimulates secretion of PRL in vivo (Clemens et al., 1978; Marti-Henneberg et al., 1980; King et al., 1985; Johnson et al., 1986; Beru and Kuhn, 1987a, b). Furthermore, treatment with 5HT agonists stimulate secretion of PRL in vivo (Lawson and Gala, 1978; Willoughby et al., 1982; Lewis and Sherman, 1985; Beru and Kuhn, 1987a,b).

Treatment with 5HT antagonists, p-chlorophenylalanine (pCPA; Donoso et al., 1971; Kordon et al., 1973/74; Gallo

et al., 1975) or cyprohaptadine (Lewis and Sherman, 1985) have little effect on basal secretion of PRL. However, 5HT antagonists reduce the ability of certain factors to stimulate secretion of PRL. For example, pCPA blocks suckling-induced secretion of PRL (Kordon et al., 1973/74); pCPA, ketanserin and ICS 205-930 block proestrus-induced secretion of PRL (Jahn and Deis, 1987, 1988), metergoline blocks stress-induced secretion of PRL (Moore et al., 1987); and cyproheptadine blocks fenfluramine-induced secretion of PRL (Lewis and Sherman, 1985). Therefore, 5HT may be involved in regulation of stimulated secretion of PRL but not basal secretion.

3. Possible sites of 5-hydroxytryptaminergic influence on prolactin. The exact mechanism whereby 5HT influences secretion of PRL is unknown but the site of action is probably not directly on the anterior pituitary gland. For example, infusion of 5HT into hypophyseal portal vessels did not influence secretion of PRL (Kamberi et al., 1971a). In addition, secretion of PRL from dispersed cells of the anterior pituitary gland treated with 5HT was either not different (Talwalker et al., 1963; Birge et al., 1970) or decreased (Padmanabhan et al., 1979) compared with controls. Furthermore, 5HT stimulation of PRL is prevented in hypothalamo-pituitary disconnected sheep (Thomas et al., 1987). Therefore, 5HT probably exerts its influence indirectly.

5-Hydroxytryptaminergic neurons project from the raphe nuclei to the SCN (Avanzino et al., 1971; Kent and Sladek, 1978; Moore et al., 1978) and since the SCN may be involved in secretion of PRL (Pasteels, 1970; Dunn et al., 1980) 5HT released from raphe nuclei to the SCN may be a mechanism whereby 5HT indirectly influences PRL. For example, microiontopheretic application of 5HT to the SCN reduces electrical activity in this nucleus (Meijer and Good, 1988). In addition, lesions of the raphe nuclei block suckling-induced increases in secretion of PRL, a mechanism that involves 5HT (Barofsky et al., 1983); indicating that 5-hydroxytryptaminergic neurons may be involved in secretion of PRL.

Alternatively, 5HT may influence DA, which, in turn, regulates PRL. For example, 5HT inhibits dopaminergic activity in the striatum (Ennis et al., 1981). 5HT may also inhibit activity of TIDA neurons, thus reducing the inhibitory influence of DA on PRL. For example, implantation of 5HT in the arcuate nucleus, the location of perikarya of TIDA neurons, reduces neuronal activity in this nucleus and increases secretion of PRL (Nishihara et al., 1986; Nishihara and Kimura, 1987). In addition, the arcuate nucleus receives 5HT innervation (Pickel et al., 1977; Kent and Sladek, 1978; Wosler et al., 1984). Therefore, 5HT may influence PRL by inhibiting TIDA neurons. Indeed, concentrations of DA in hypophyseal portal blood (from TIDA neurons) were decreased in rats

receiving intraventricular 5HT (Pilotte and Porter, 1981). In explants of rat brain, 5HT inhibits activity of tyrosine hydroxylase, the rate limiting enzyme in synthesis of DA (Devau et al., 1987). This inhibition was dose dependent and reversible (Devau et al., 1987).

These two mechanisms may be connected, because axons from the SCN project to the arcuate nucleus (Swanson and Cowan, 1975; Rusak and Zucker, 1979). Therefore, 5HT may affect secretion of PRL by altering activity of SCN which, in turn, reduces dopaminergic activity in the arcuate nucleus.

A third alternative is that 5HT may influence secretion of PRL by positive stimulation. For example, Clemens et al. (1978) reported that 5HT-induced secretion of PRL was greater than DA-inhibition of PRL. They concluded that 5HT stimulated secretion of PRL by causing release of a PRL-releasing factor not by a reduction of TIDA neuronal activity (Clemens et al., 1978).

4. Summary. Tryptophan is converted to 5HT in 5-hydroxytryptaminergic neurons. Tryptophan hydroxylase, the rate limiting enzyme in 5HT synthesis, can be controlled by substrate availability or end-product inhibition. 5HT stimulates secretion of PRL but not as a direct effect on the anterior pituitary gland. The indirect mechanism whereby 5HT stimulates PRL is unknown. However, 5HT may inhibit SCN (and)or TIDA neuronal activity, which in turn,

increases secretion of PRL. Alternatively, 5HT may stimulate a prolactin-releasing factor which stimulates secretion of PRL. Thus, photoperiod may induce changes in 5HT activity at the SCN and(or) the arcuate nucleus which in turn may alter secretion of PRL. Indeed, as mentioned previously, photoperiod can alter concentrations of 5HT and 5HIAA/5HT in hamsters (Benson, 1987).

Chapter 1

Alterations in Concentrations of Dihydroxyphenylacetic Acid in Rats Euthanized with Sodium Pentobarbital

Introduction

An objective of this dissertation was to determine if exposure to photoperiods that alter concentrations of PRL in cattle alter activity of TIDA and 5HT neurons. Accurate measurement of activity of these neurons requires rapid removal of the brain followed by dissection of the MBH /pituitary stalk (PS), which contains these neurons. In rats, the brains and pituitary gland are quickly removed following decapitation but, because of body size this method is not appropriate for cattle. Furthermore, the traditional method of slaughtering cattle (i.e., stunning with a captive bolt and exsanguination) is unacceptable because it activates the hypothalamo-pituitary system (Cooper et al., 1986) and damages the hypothalamus (Dennis et al., 1988).

Additional requirements of this series of experiments were that bulls be euthanized without stress and without altering ambient temperature. Stress increases secretion of PRL (Tucker, 1971) and restraint stress-induced increases in PRL are associated with altered TIDA and 5HT neuronal activity (Demarest et al., 1985a). In addition, ambient temperature acutely affects serum concentrations of PRL in cattle (Tucker and Wettemann, 1976). Furthermore, cold temperatures block photoperiod-induced increases in serum concentrations of PRL (Peters and Tucker, 1978). Therefore, stress or changing ambient temperatures at

slaughter may mask photoperiod-induced effects on serum concentrations of PRL or TIDA and (or) 5HT neuronal activities. Thus, a method of euthanizing cattle in these studies was needed in which animals could be killed without stress and at constant ambient temperature.

Sodium pentobarbital-based mixtures, available commercially are used for euthanizing domestic animals (McDonald et al., 1978). Large doses of pentobarbital, given intravenously, euthanizes animals rapidly by direct action on the medulla causing depression of the respiratory center and cessation of heart action (McDonald et al., 1978). Since animals die rapidly with little stress and animals can be killed in their stalls in our environmentally-controlled chamber, sodium pentobarbital met the criteria for a euthanizing agent needed for these experiments. However, the effects of a commercially available euthanizing agent, Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI), or sodium pentobarbital on TIDA neuronal activity were unknown. Therefore, the primary objective of this experiment was to determine the effects of sodium pentobarbital on activities of TIDA neurons. Since localization of important areas in the hypothalamus of TIDA neuronal activities have been elucidated in rats (Moore et al., 1985), rats were utilized in this experiment. Specifically, the objectives were: to compare concentrations of DOPAC and DA in selected brain

regions of rats that were euthanized with Fatal Plus, sodium pentobarbital or decapitation, and 2) to compare the effects of immobilization stress on DOPAC and DA in rats euthanized with sodium pentobarbital or decapitation.

Materials and Methods

Animals. Male Long-Evans rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 240 to 270 g were maintained in a temperature- (22°C) and photoperiod- (lights on between 0600 and 1800 h) controlled environment. Food (Wayne Lablox, Continental Grain Company, Chicago, IL) and water were provided ad libitum. Euthanizing agents were infused iv. Three d before an experiment a polyethylene cannula was implanted into a jugular vein in rats that received iv injections. Before surgery, rats were anesthetized with ether.

Treatments. In a preliminary study (Experiment 1) eight rats were euthanized with an iv injection of Fatal Plus (equivalent to 85 mg sodium pentobarbital/kg body weight; a dose that kills rats within 2 sec) and eight rats were untreated and served as controls.

In subsequent studies (Experiments 2 and 3) Fatal Plus or an aqueous solution of its active ingredient, sodium pentobarbital (388 mg/ml; pH 8.5), were infused iv as indicated in table legends.

Tissue collection, preparation and analysis. Following treatment with Fatal Plus, sodium pentobarbital or no treatment of controls, rats were decapitated and brains and pituitary glands were removed and frozen on aluminum foil placed directly on dry ice. Frontal sections (1 mm) were prepared in a cryostat (-9°C) and selected brain regions were dissected according to modifications (Lookingland and Moore, 1985) of the method of Palkovits (1973). Tissues were placed in 60 μl of .1 M phosphate citrate buffer (pH 2.5) containing 15% methanol and stored at -20°C until assayed. At the time of assay each sample was sonnicated (3 sec), centrifuged, and contents of DA and DOPAC in the supernatant were determined by high performance liquid chromatography (HPLC) coupled to an electrochemical detector (Chapin et al., 1986). Tissue pellets were dissolved in 1.0 N NaOH and assayed for protein (Lowry et al., 1951). Statistical analysis of differences between groups were analyzed by the two-tailed Students' t-test (Gill, 1978).

Results

Experiment 1. It was initially planned to measure the concentrations of DA and DOPAC in the median eminence of cattle that were euthanized with Fatal Plus. To determine if this euthanizing agent per se altered the concentrations of these compounds a preliminary experiment was conducted

in rats. Relative to decapitated rats, rats euthanized with Fatal Plus had greater ($P < .01$) concentrations of DOPAC (8.5 ± .5 vs 16.9 ± 1.6 ng/mg protein) and lower ($P < .01$) concentrations of DA (110.5 ± 4.6 vs 88.8 ± 3.8 ng/mg protein) in the median eminence.

Experiment 2. Since results of Experiment 1 were unexpected, it was decided to determine if the neurochemical effects of Fatal Plus were associated with its content of sodium pentobarbital. Accordingly, concentrations of DOPAC and DA determined in selected brain regions were compared in rats that were decapitated or euthanized with either Fatal Plus or with sodium pentobarbital. Concentrations of DOPAC and DA in the median eminence, striatum and SCN are summarized in Table 1. Similar to the results in Experiment 1, Fatal Plus increased concentrations of DOPAC 52% and reduced concentrations of DA 28% in the median eminence. Relative to decapitation sodium pentobarbital was without effect on concentrations of DA but increased concentrations of DOPAC 49% in the median eminence. It was concluded that increased concentrations of DOPAC in the median eminence of rats euthanized with Fatal Plus in Experiment 1 were due to its content of pentobarbital, whereas the reduction in DA after Fatal Plus was probably due to an ingredient other than pentobarbital. The effects of Fatal Plus and pentobarbital on concentrations of DA and DOPAC were

Table 1.

Concentrations of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) in brain regions rats euthanized with Fatal Plus, pentobarbital or decapitation.

Brain Region	Decapitation		Fatal Plus		Pentobarbital	
	DA	DOPAC	DA	DOPAC	DA	DOPAC
Median eminence	83.3 ± 6.0	5.4 ± .4	60.1 ± 4.3	11.3 ± .8[b]	76.8 ± 4.2	10.5 ± 1.3[b]
Striatum	8.5 ± 2.3	22.9 ± .9	86.1 ± .8	27.9 ± 1.3	80.9 ± 1.6	22.4 ± .7
Suprachiasmatic nucleus	4.5 ± .5	1.0 ± .1	3.7 ± .3	1.0 ± .1	4.9 ± .4	1.1 ± .1

Male rats were decapitated or euthanized with an iv injection of Fatal Plus (85 mg sodium pentobarbital/kg body weight) or sodium pentobarbital (85 mg/kg body weight) prior to decapitation. Each value (ng/mg protein) represents the mean ± standard error of 7 or 8 animals.

[a]Lobes of pituitary gland.

[b]Different from decapitated controls.

restricted to the median eminence; neither preparation altered concentrations of DOPAC or DA in the striatum or SCN (Table 1).

Experiment 3: Because pentobarbital increased concentrations of DOPAC in the median eminence it was important to determine if stimuli that normally influence this neurochemical index of TIDA neurons are altered in rats euthanized with sodium pentobarbital. Therefore, it was decided to determine if the ability of restraint stress to reduce concentrations of DOPAC in the median eminence was masked in rats euthanized with sodium pentobarbital. The results of this experiment are summarized in Table 2.

Consistent with the results in Experiment 2 (Table 1) concentrations of DOPAC in the median eminence of rats euthanized with sodium pentobarbital were increased compared with DOPAC in rats that were decapitated. Nevertheless, 15 min of physical restraint reduced concentrations of DOPAC in the median eminence of decapitated and pentobarbital-euthanized rats. Fifteen min of restraint did not influence DOPAC or DA in the striatum.

Discussion

Consistent with previous reports (Umezu and Moore, 1979), concentrations of DOPAC relative to those of DA are lower in the median eminence than in the striatum.

Table 2.

Effects of stress on concentrations of dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) in the median eminence and striatum of rats euthanized with pentobarbital or decapitation.

Method of Euthanasia	Median Eminence		Striatum	
	DA	DOPAC	DA	DOPAC
Decapitation				
Control	104.3 ± 4.9	8.1 ± .4	106.1 ± 2.2	31.5 ± .9
Stress	87.7 ± 8.9	4.4 ± .2[a]	112.2 ± 2.8	32.7 ± 1.3
Pentobarbital				
Control	103.6 ± 10.7	12.2 ± 1.0[b]	112.9 ± 3.6	28.3 ± 1.2
Stress	73.1 ± 9.1	7.8 ± .4[a]	113.1 ± 3.8	32.1 ± 2.0

Stressed rats were anesthetized with diethylether (2 min), restrained in the supine position, and sacrificed 15 min later. Control rats were anesthetized with diethylether (2 min), returned to their cages, and sacrificed 15 min later. Rats were either decapitated or euthanized with an iv injection of sodium pentobarbital (85 mg/kg body weight) prior to decapitation. Each value (ng/mg protein) represents the mean ± standard error of 7 or 8 animals.

[a]Different from unstressed controls ($P < .01$).

[b]Different from decapitated animals ($P < .01$).

However, concentrations of DOPAC in the median eminence of animals euthanized with Fatal Plus or sodium pentobarbital were greater than DOPAC in decapitated animals. Concentrations of DA were reduced in animals injected with Fatal Plus but not with sodium pentobarbital, suggesting that this effect may be due to some constituent of Fatal Plus other than sodium pentobarbital. Neither drug altered concentrations of DA or DOPAC in the striatum, arcuate nucleus, nucleus accumbens or intermediate or neural lobes of the pituitary gland, suggesting that these effects are selective for TIDA neurons in median eminence.

Physical restraint reduces rates of turnover and synthesis of DA and concentrations of DOPAC in the median eminence of decapitated rats (Demarest et al., 1985a; Lookingland et al., 1987b); suggesting that this stressful manipulation reduces TIDA neuronal activity. Similarly in the current study, 15 min of restraint reduced concentrations of DOPAC in the median eminence of decapitated and pentobarbital-euthanized rats, but did not alter concentrations of DOPAC in the striatum.

Results of the present study reveal that sodium pentobarbital-induced euthanasia specifically increases concentrations of DOPAC in the median eminence. This effect is rapid (within 25 sec; from beginning of injection to removal of brain) and could represent an increase in the metabolism of DA associated with or independent of an increase in TIDA neuronal activity. Alternatively, sodium

pentobarbital could decrease a post-mortem decline in concentrations of DOPAC in the median eminence of decapitated rats. Regardless of the mechanism, changes in concentrations of DOPAC in the median eminence associated with restraint stress-induced change in TIDA neuronal activity were not masked in animals euthanized with sodium pentobarbital. Therefore, pharmacological-induced changes in concentrations of DOPAC in the median eminence (as an estimation of TIDA neuronal activity) can be detected in rats euthanized with sodium pentobarbital. Therefore, it was decided to test if similar neurochemical measurements made in cattle euthanized with sodium pentobarbital were related to activity of TIDA neurons.

Chapter 2

Prolactin Regulation of Tuberoinfundibular Dopaminergic Neurons in Bull Calves

Introduction

DA inhibits secretion of PRL in cattle in vitro (Padmanabhan et al., 1979). In addition, treatment with a DA agonist, bromocriptine, reduced concentrations of PRL in cattle in vitro and in vivo (Smith et al., 1974). Conversely, in cattle treated with drugs that disrupt synthesis of DA (e.g., α-methyltyrosine) or interfere with action of DA at the anterior pituitary gland (e.g., haloperidol) serum concentrations of PRL are increased in vivo (Benoit, 1987). Location and characteristics of dopaminergic neurons that regulate secretion of PRL in mammals are based on studies conducted with the rat. The DA primarily responsible for the tonic inhibition of secretion of PRL in the rat is released from terminals of TIDA neurons located in the median eminence in the MBH (Gudelsky, 1981). DA released from TIDA neurons is transported in the hypophyseal portal blood to the anterior pituitary gland where it binds to receptors on the lactotropes and inhibits secretion of PRL (Ben-Jonathan, 1985). In the bovine hypothalamus DA has been detected in high concentrations in the infundibulum and PS (Cooper et al., 1986), but it is not known if these dopaminergic neurons regulate secretion of PRL.

In rats, DA released from TIDA neurons inhibits secretion of PRL and PRL, in turn, regulates activity of TIDA neurons (Hokfelt and Fuxe, 1972; Gudelsky et al.,

1976; Annunziato and Moore, 1978). Pharmacological manipulations that increase (e.g., DA antagonists) or decrease (e.g., DA agonists) serum concentrations of PRL cause corresponding changes in activity of TIDA neurons (Moore, 1987). Furthermore, systemic (Hokfelt and Fuxe, 1972; Gudelsky et al., 1976) and intracerebroventricular (Annunziato and Moore, 1978; Johnston et al., 1980) injections of PRL increase activity of TIDA neurons and decrease endogenous secretion of PRL (Selmonoff and Gregerson, 1984), suggesting that PRL regulates its own secretion by altering the level of inhibitory dopaminergic tone.

In the present study, location of TIDA neurons in cattle was determined by: 1) measuring the distribution of DA in selected areas of the hypothalamus, and 2) examining the ability of elevated serum concentrations of PRL to regulate the activity of these neurons.

Materials and Methods

Experiment 1. Five prepubertal Holstein bull calves (approximately 8 wk of age) were euthanized with sodium pentobarbital [Sigma Chemical Company, St. Louis, MO; iv 85 mg/kg body weight in sterile water (388 mg/ml, pH 8.0)]. Skin was excised along the forehead, just above the eyes and from the eyes to the pole on both sides of the head.

The skull was then cut with a reciprocating saw to expose the dorsal portion of the brain. The optic nerve and PS were cut at their distal junctions with the dura and brains were removed and placed on a petri dish over ice. A block of tissue (Figure 1A) containing the MBH and PS was dissected with a scalpel blade, placed with the ventral surface up on aluminum foil and frozen with dry ice. Frontal sections (1 mm) beginning caudal to the optic chiasm (Figure 1B) were prepared on a cryostat (-9°C) and the PS and MBH were dissected from these frozen sections with a scalpel blade (Figure 1C). In addition, a 2000 μ punch (12 gauge inner diameter) was used to remove the SCN from areas indicated in Figure 1A. Although TIDA neurons are not located in the SCN, this area was dissected and analyzed for a comparison with the MBH and PS.

Analysis of DA and DOPAC. DA, DOPAC and protein were determined in SCN, MBH and within each 1 mm section of PS as described in Chapter 1.

Statistical analysis. DOPAC and DA data were analyzed by analysis of variance and differences among areas within the hypothalamus and PS were determined by Bonferroni-t procedure (Gill, 1978).

Experiment 2. Sixteen prepubertal Holstein bull calves (approximately 8 wk of age) were assigned randomly to one of two treatments. Eight bulls were injected sc with haloperidol [Sigma; 1 mg/kg body weight (2.5 mg

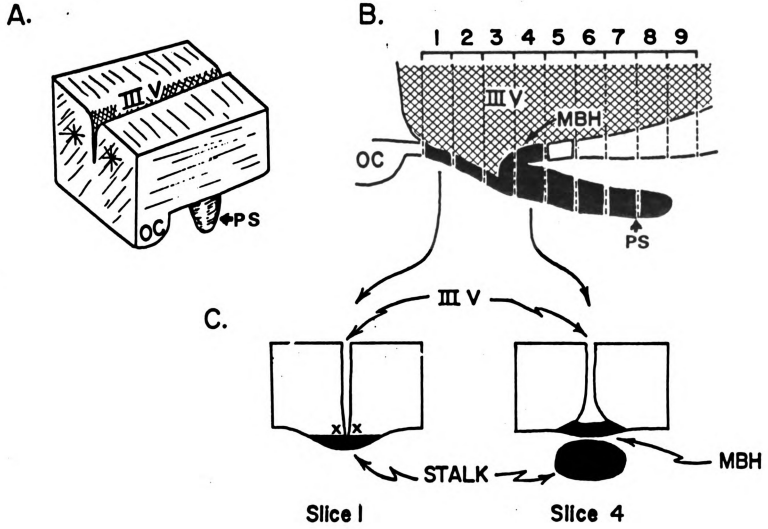
Figure 1. Schematic diagram of the dissection of the infundibular-pituitary stalk (PS), mediobasal hypothalamus (MBH) and suprachiasmatic nucleus (SCN) of bull calves.

A. Dissected block of hypothalamus and attached PS; III V = third ventricle OC = optic chiasm; * = area of SCN.

B. Midsaggital view depicting the location of sections (1 through 9) of PS.

C. Frontal views showing the first and fourth sections; x = area of arcuate nucleus.

Dark areas represent the location of the MBH and PS that were dissected for subsequent analysis.


A.
III V
OC
PS
B.
1 2 3 4 5 6 7 8 9
III V
MBH
OC
PS
C.
III V
STALK
MBH
Slice 1
Slice 4

haloperidol/ml of .3% tartaric acid)] every 6 h for 24 h. This dose maintains elevated serum concentrations for 6 to 8 h (S.A. Zinn and H.A. Tucker, unpublished observations). Eight uninjected bulls were controls.

Bulls were housed in individual stalls in light (24L:0D)- and temperature (20 ± 2°C)-controlled chambers (four bulls/chamber). Cool-white fluorescent tubes at an average intensity of 400 lux (measured 1 M from the floor) were utilized as the source of light. Bulls were given 1 kg of a commercial calf grain (Startina, Purina Mills, St. Louis, MO), daily at 0800 and 1700 h. Water was provided ad libitum.

One d before start of treatments, each bull was fitted with a polyvinyl cannula in a jugular vein. The next day blood was collected and discarded every 10 min from 0700 to 0920 h, to accustom bulls to sampling procedures. Beginning at 0930 h blood was sampled every 15 min for 1 h before the start of haloperidol injections and then every 2 h for the next 24 h. One sample was taken just before euthanasia (60 min after the last haloperidol injection). Blood samples were stored at 20°C for 4 to 6 h and then overnight at 4°C. Samples were centrifuged, sera decanted and stored at -20°C until assayed for PRL (Koprowski and Tucker, 1971). Bulls were euthanized with sodium pentobarbital (iv, 85 mg/kg body weight) 60 min after the last haloperidol injection. Following euthanasia, the brain was dissected, frozen and concentrations of DOPAC, DA

and protein were determined as described in Experiment 1 (Chapter 2). Changes in concentrations of DOPAC and ratio of DOPAC to DA were used as estimates of dopaminergic activity in the PS (Lookingland et al., 1987b).

Statistical analysis. Treatment differences in concentrations of DOPAC and DA were compared by analysis of variance (Gill, 1978). PRL data were analyzed by split-plot analysis of variance with repeated measurement (Gill and Hafs, 1971).

Experiment 3. The experimental design of Experiment 2 (Chapter 2) was repeated with an additional 16 prepubertal bull calves (approximately 8 wk of age). Treatments, housing, cannulations and blood sampling were as described in Experiment 2 (Chapter 2). However, 15 min before euthanasia an additional blood sample was collected and then bulls were injected iv with an aromatic L-amino acid decarboxylase inhibitor [NSD 1015 (3-hydroxybenzyl-hydrazine, Sigma) 25 mg/kg body weight (100 mg NSD 1015/ml .9% saline)]. NSD 1015 inhibits conversion of DOPA to DA and 5HTP to 5HT, and acumulation of DOPA (the immediate precursor of DA) and 5HTP (the immediate precursor of 5HT) over time reflects in vivo rate of DA and 5HT synthesis, respectively (Carlsson et al., 1972). Since the synthesis and release of neurotransmitters are tightly coupled in these neurons, the rate of DA and 5HT synthesis may be used as indices of DA (Demarest and Moore, 1980) and 5HT

neuronal activity (Duda and Moore, 1985), respectively.

Following euthanasia, the brain was dissected, frozen and concentrations of DOPA, DA and protein were determined as described in Experiment 1 (Chapter 2). Concentrations of 5HTP and 5HT were determined by HPLC concurrently with DOPA and DA (Chapin et al., 1986).

Statistical analysis. Treatment differences in concentrations of DOPA, 5HTP, DA and 5HT were compared by analysis of variance (Gill, 1978). PRL data were analyzed by split-plot analysis of variance with repeated measurement (Gill and Hafs, 1971).

Experiment 4. To determine if haloperidol-induced increases in TIDA neuronal activity were due to increased prolactin per se and to determine if these changes in TIDA activity could be maintained for 9 d a third experiment was conducted. The duration of Experiment 4 was 9 d. Eighteen prepubertal Holstein bull calves (approximately 10 wk of age) were randomly assigned to one of three treatments. Six bulls were infused with PRL for 9 d and six bulls were uninfused for 8 d and then infused with PRL for the last 25 h and six uninfused bulls were controls. Housing, feeding and photoperiod was a described in Experiment 2 (Chapter 2).

One d before (d -1) the 9 d infusion began, each bull was fitted with a polyvinyl cannula in a jugular vein. In addition, bulls receiving PRL infusions were fitted with a polyvinyl cannula in the contralateral vein one d before

start of infusion (i.e., d -1 for the 9 d infusion group and d 7 for the 25 h infusion group).

Prolactin (USDA b-1) for infusion was prepared in a sterile solution containing .1% bovine serum albumin (BSA) in .85% saline at a concentration of 1 mg PRL/ml saline. Bulls received pulses of PRL every 3.75 min (.5 ml/h) using a AS-2BH Autosyringe infusion pump (Autosyringe, Inc., Hooksett, NH). Infusions began at 1230 h on d 0 for bulls receiving PRL for 9 d and at 0900 h on d 8 foor bulls receiving PRL for 25 h.

Beginning on d 0, blood was collected and discarded every 10 min from 0930 to 1115 h, to accustom bulls to sampling procedures. Beginning at 1130 h blood samples were collected every 15 min for 1 h and every 6 h until 0800 h on d 8. Beginning at 0800 h on d 8 blood samples were collected every 15 min for 1 h and then every 4 h for the next 24 h. In addition, one sample was taken just prior to infusion of NSD 1015 (0945 h; d 9) and one sample was taken just prior to euthanasia (1000 h; d 9). Blood samples were stored at 20°C for 4 to 6 h and then overnight at 4°C. Samples were centrifuged and sera were decanted and stored at -20°C until assayed for PRL as described in Experiment 2 (Chapter 2).

Beginning at 0945 h on d 9 all bulls were treated with NS 1015 as described in Experiment 2 (Chapter 2). Bulls were then euthanized, tissue was collected and

prepared and concentrations of DOPA, DA and protein were determined as described in Experiment 1 (Chapter 2).

Statistical analysis. DOPA and DA data were analyzed by analysis of variance and differences between treatment means were compared by Bonferoni t-test (Gill, 1978). PRL data were analyzed by split-plot analysis of variance with repeated measurement (Gill and Hafs, 1971) and differences between treatments were compared by Bonferoni t-test (Gill, 1978).

Results

Experiment 1. Among bulls, the number of slices per animal and the concentrations of DA within each slice varied. But greatest concentrations of DA were usually in the PS (slice 2, 3, 4 or 5; Table 3). Concentrations of DOPAC within the PS paralleled concentrations of DA (Table 4). Concentrations of DA and DOPAC averaged across all slices of PS were at least 2.5-fold greater ($P < .05$) than concentrations in the SCN or MBH (Table 5). Therefore, changes in dopaminergic activity in the PS became the focus of Experiments 2, 3 and 4 (Chapter 2).

Experiment 2. Haloperidol, injected every 6 h increased ($P < .05$) serum concentrations of PRL, relative to control (87.3 ± 6.4 vs 15.0 ± 1.8 ng/ml; patterns of serum concentrations of PRL in these calves are similar to those shown in Figure 2).

Table 3.

Concentrations of Dopamine in Sections of Pituitary Stalk of Bull Calves.

Slices[a]		Bull 1	2	3	4	5
		pg/μg protein				
1	Proximal	3.06	1.90	1.71	5.06	1.72
2		10.00	5.42	6.99	7.86	5.52
3		12.05	7.19	9.00	8.47	12.43
4		10.40	8.81	10.23	8.36	10.36
5		9.65	5.31	10.68	7.45	10.11
6		8.45	5.62	5.04	4.51	6.58
7		6.83	6.84	1.83		3.23
8		8.83	8.90			2.47
9	Distal	3.78				

[a]One mm frontal slices beginning at the junction of the pituitary stalk and infundibular recess (proximal) and ending at the junction of the pituitary stalk and anterior pituitary gland (distal).

Table 4.

Concentrations of Dihydroxyphenylacetic Acid in Slices of Pituitary Stalk of Bull Calves.

Slices[a]		Bull 1	2	3	4	5
		pg/μg protein				
1	Proximal	2.11	1.56	.61	2.28	.77
2		4.88	3.76	2.77	3.02	1.92
3		5.08	4.08	3.31	3.59	4.17
4		3.84	4.86	5.34	2.88	3.76
5		3.60	2.61	4.13	2.73	3.40
6		2.92	2.28	2.29	1.78	2.05
7		1.44	3.24	.56		.73
8		1.94	2.26			.83
9	Distal	.54				

[a]One mm frontal slices beginning at the junction of the pituitary stalk and infundibular recess (proximal) and ending at the junction of the pituitary stalk and anterior pituitary gland (distal).

Table 5.

Concentrations of Dopamine (DA) and Dihydroxyphenylacetic Acid (DOPAC) in Brain Regions of Bull Calves[a].

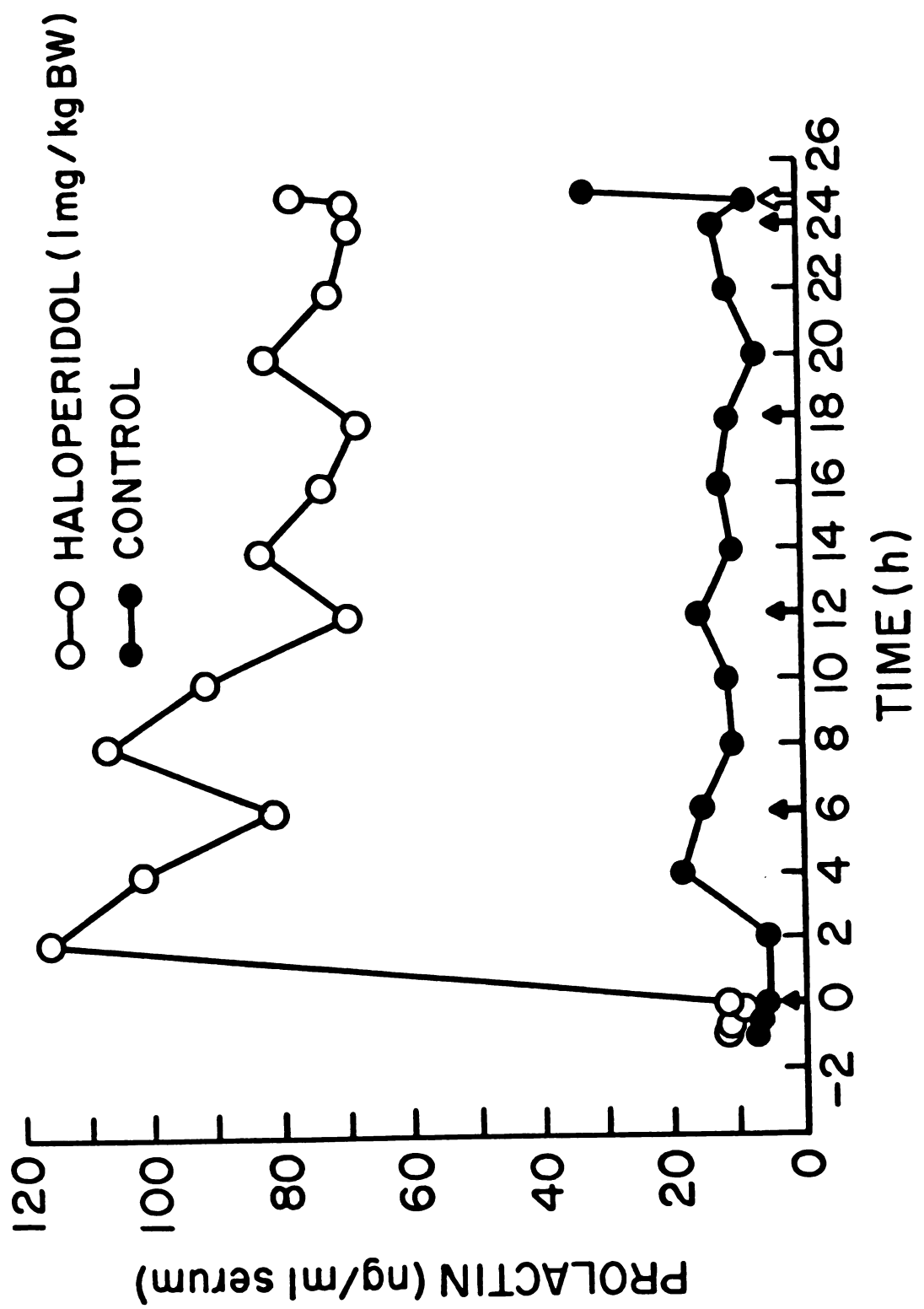
Region	DA (pg/μg protein)	DOPAC (pg/μg protein)
Pituitary stalk[b]	6.9 ± .3[c]	2.72 ± .13[c]
Suprachiasmatic nucleus	1.6 ± .4	.42 ± .03
Mediobasal hypothalamus	1.9 ± .4	.61 ± .03

[a]Values represent means (± SE) for DA and DOPAC. Average of five bull calves.

[b]Average of all slices of pituitary stalk.

[c]Means from pituitary stalk differ from means of other regions ($P < .05$).

Figure 2. Concentrations of prolactin in serum of bull calves injected with haloperidol (▲; 1 mg/kg body weight) every 6 h (○) or uninjected controls (●). All calves received NSD 1015 (25 mg/kg body weight; △) 15 min before euthanasia. Each point represents the mean of 7 or 8 samples. Standard error = 5.2 ng/ml for haloperidol injected bulls and 1.6 ng/ml for uninjected controls.


HALOPERIDOL (1 mg/kg BW)
CONTROL
PROLACTIN (ng/ml serum)
120
100
80
60
40
20
0
-2
0
2
4
6
8
10
12
14
16
18
20
22
24
26
TIME (h)

Similar to results in Experiment 1 (Chapter 2), the number of slices of PS per bull and the concentrations of DOPAC and DA within each slice varied. Concentrations of DOPAC and DA, averaged across all slices of PS, are summarized in Table 6. Averaged across all slices of PS, haloperidol did not affect concentrations of DOPAC or DA or ratios of DOPAC to DA in PS of bull calves. However, when the statistical analysis was done on the individual section with the greatest concentration of DA or on the second through fourth slices regardless of concentration of DA, the ratio of DOPAC to DA was increased ($P < .05$) two-fold in bulls injected with haloperidol compared with controls. Although, changes in the ratio of DOPAC to DA following injections of haloperidol were in the anticipated direction, the magnitude of response was small. Therefore, in Experiments 3 and 4 accumulation of DOPA following a decarboxylase inhibitor NSD 1015 was used as the index of dopaminergic activity is PS of bull calves.

Experiment 3. Haloperidol, injected every 6 h, increased ($P < .05$) serum concentrations of PRL, relative to control (83.1 ± 5.2 vs 13.3 ± 1.6 ng/ml; Figure 2). Injection of NSD 1015 increased ($P < .05$) concentrations of PRL within 15 min in control animals but not in haloperidol-treated bulls (Figure 2).

Similar to results in Experiment 1 (Chapter 2), the number of slices of PS per bull and the concentrations of DA within each slice varied. Concentrations of DOPA and DA

Table 6.

Effects of 25 h of exposure to haloperidol on concentrations of DOPAC in pituitary stalk of bull calves[a].

	Haloperidol	Control
DOPAC[b]	4.62 ± .2	4.64 ± .5
DA[b]	7.41 ± .7	9.85 ± 1.3
DOPAC/DA	.62 ± .12	.48 ± .10

[a]Values represent means of 7 or 8 bulls (± SE) for dihydroxyphenylacetic acid (DOPAC; pg/μg protein) and dopamine (DA; pg/μg protein).

[b]Within treatment values are averages of all slices of pituitary stalk.

averaged across all slices of PS, are summarized in Table 7. Concentrations of DOPA were increased and concentrations of DA were reduced in bulls injected with haloperidol when compared with controls. Thus, the ratio of DOPA to DA increased in haloperidol-injected bulls (Table 7). Inferences did not change if the data were based on the average of all slices or in the individual slices with the highest concentrations of DA and DOPA. Haloperidol did not affect concentrations of 5HT or its precursor, 5HTP, in PS (Table 8).

Experiment 4. Averaged over the duration of infusion of PRL, concentrations of PRL were increased ($P < .05$) in bulls infused for 25 h (204.1 ± 11.8 ng/ml) or 9 d (183.3 ± 10.6 ng/ml) compared with uninfused controls (13.9 ± 1.0 ng/ml; averaged over the 9-d duration of the experiment; Figure 3). Averaged over the non-infusion periods serum concentrations of PRL in bulls infused for 25 h (samples collected during the 8 d before starting infusion) and for 9 d (sample collected during the 1 h before starting infusion) averaged 14.7 ± 1.3 ng/ml and 11.8 ± 1.8 ng/ml, respectively and were not different ($P > .10$) from the control concentration (Figure 3). Injection of NSD 1015 increased concentrations of PRL within 15 min in controls but not in bulls infused with PRL (Figure 3). Within each day there appears to be a rhythm of secretion of PRL with a peak at 2200 or 0400 h and a nadir 12 h later. However,

Table 7.

Effect of 25 h of exposure to haloperidol on accumulation of DOPA activity in pituitary stalk of bull calves[a].

	Haloperidol	Control
DOPA[b]	1.57 ± .16[c]	.98 ± .09
DA[b]	6.80 ± .64[c]	10.71 ± .54
DOPA/DA	.23 ± .03[c]	.09 ± .01

[a]Values represent means of 7 or 8 bulls (± SE) for dihydroxyphenylalanine (DOPA; pg/μg protein) and dopamine (DA; pg/μg protein).

[b]Within treatment values are averages of all sections of pituitary stalk.

[c]Means from haloperidol-treated bulls differ from means from controls ($P < .05$).

Table 8.

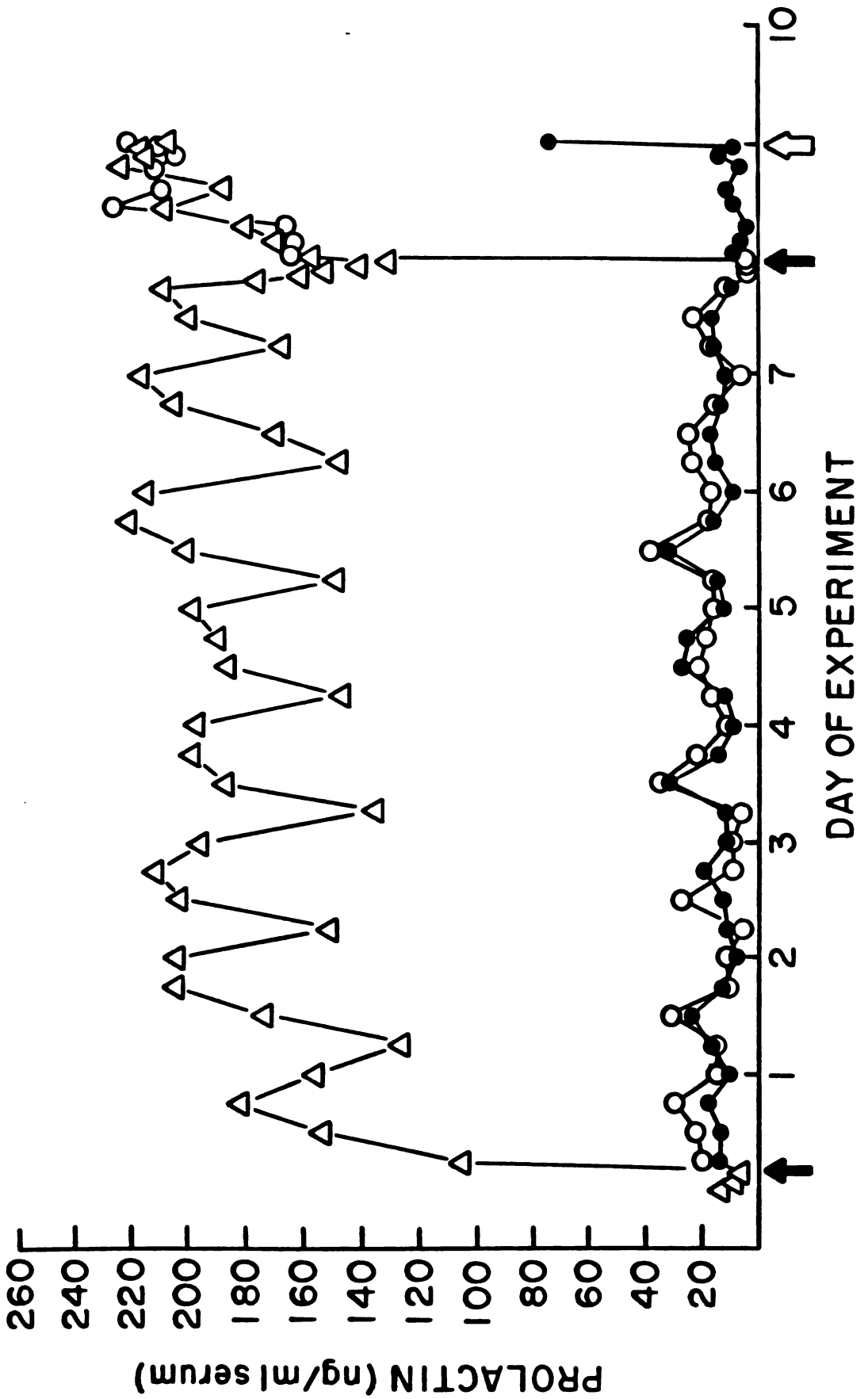
Effects of 25 h of exposure to haloperidol on accumulation of 5HTP in pituitary stalk of bull calves[a].

	Haloperidol	Control
5HTP[b]	.29 ± .03	.34 ± .03
5HT[b]	5.45 ± .97	5.20 ± .53
5HTP/5HT	.06 ± .01	.07 ± .01

[a]Values represent means of 7 or 8 bulls (± SE) for 5-hydroxytryptophan (5HTP; pg/μg protein) and 5-hydroxytryptamine (5HT; pg/μg protein).

[b]Within treatment values are averages of all sections of pituitary stalk.

Figure 3. Concentrations of prolactin in serum of prepubertal bull calves infused with prolactin (USDA b1; 12.0 mg/d) for 25 h (○), 9 d (▲), and non-infused controls (●). Beginning of infusion indicated by solid arrows (⬆). All animals received NSD-1015 (iv; 25 mg/kg BW; ⇧) 15 min before euthanasia. Each point represents the mean of 5 or 6 samples. Pooled standard errors = 4.3 ng/ml for periods of non-infusion and 11.2 ng/ml for periods of infusion.


PROLACTIN (ng/ml serum)
260
240
220
200
180
160
140
120
100
80
60
40
20
1
2
3
4
5
6
7
10
DAY OF EXPERIMENT

these times correspond to the maximum and minimum temperatures within the chambers.

Concentrations of DOPA and DA, averaged across all slices of the PS, are summarized in Table 9. Infusion of PRL increased concentrations of DOPA in bulls infused for 25 h or 9 d compared with controls, but infusion of PRL did not affect concentrations of DA. However, the ratio of DOPA to DA increased in bulls infused with PRL for 25 h or 9 d compared with controls. Concentrations of DOPA and DA and ratio of DOPA to DA were not different between bulls infused for 25 h or 9 d (Table 9).

Discussion

Although little is known regarding the location of terminals of TIDA neurons in the bovine hypothalamus, Cooper et al. (1986) reported that in steers concentrations of DA and DOPAC in the infundibulum and infundibular stalk were greater than in the anterior pituitary gland and zona tuberalis. In agreement, the results from the present study indicate that average concentrations of DA and DOPAC in the PS of bull calves were two to three times greater than concentrations in the MBH. Thus, TIDA neurons in the bovine hypothalamus most likely terminate in the PS. These conclusions are consistent with previous reports that the PS also contains the greatest concentrations of 5HT (Piezzi

Table 9.

Effects of infusion of prolactin for 25 h or 9 days on accumulation of DOPA in pituitary stalk of bull calves[a].

	DOPA[b]	DA[b]	DOPA/DA
Control	1.69[d]	11.56[d]	.15[d]
25 h	3.17[e]	9.7[d]	.33[e]
9 days	3.83[e]	10.13[d]	.38[e]
SE[c]	.47	1.80	.04

[a]Values represent means of six bulls for dihydroxyphenylalanine (DOPA; pg/μg protein) and dopamine (DA; pg/μg protein).

[b]Within treatment values are averages of all sections of pituitary stalk.

[c]Pooled standard error.

[d,e]Means with a different superscript within a column differ ($P < .05$).

et al., 1970) and GnRH (Kizer et al., 1976; Estes et al., 1977).

Haloperidol, a dopamine antagonist, blocks D_2 dopamine receptors on the anterior pituitary gland thereby reducing the inhibitory influence of DA on secretion of PRL (Gunnet and Moore, 1988). Similar to previous reports in rats (Dickerman et al.,1974), humans (Poland and Rubin, 1981) and cattle (Benoit et al., 1987), haloperidol increased serum concentrations of PRL in bulls in the present study. In addition, elevated serum concentrations of PRL were maintained with an injection of haloperidol every 6 h, indicating that bulls do not become refractory to repeated injections of haloperidol within 25 h.

NSD 1015 blocks synthesis of DA (Demarest et al., 1979) and reduces concentrations of DA in hypophyseal portal blood (Reymond and Porter, 1982) which results in increased serum concentrations of PRL (Demarest et al., 1984). Similarly in the present study injection of NSD 1015 acutely increased serum PRL in controls indicating that DA inhibits secretion of PRL in cattle. Since treatment with haloperidol blocks the action of DA at the pituitary gland and bulls infused with PRL already had elevated PRL, the acute effect of NSD 1015 on PRL in these animals was not observed. These results are consistent with an inhibitory role of TIDA neurons in the regulation of secretion of PRL in cattle.

The accumulation of DOPA following NSD 1015 administration is an index of the in vivo rate of dopamine synthesis in the rat brain (Carlsson, et al., 1972). Alterations in impulse flow in TIDA neurons produce corresponding changes in the rate of DOPA accumulation in the median eminence (Gunnet et al., 1987), and this measure provides a good index of TIDA neuronal activity (Demarest, et al., 1979). In the present study, procedures that increased serum concentrations of PRL (i.e., haloperidol; infusion of PRL) increased accumulation of DOPA in the PS of bull calves in 1 d. These results are consistent with stimulatory action of PRL on TIDA neurons in the rat (Hokfelt and Fuxe, 1972; Gudelsky et al., 1976; Annunziato and Moore, 1978; Johnston et al., 1980; Moore et al., 1987). Therefore, the rate of accumulation of DOPA in the PS reflects activity of TIDA neurons in cattle. Because of the smaller magnitude of response to PRL, changes in concentrations of DOPAC may not be a sensitive enough measure of change in dopaminergic activity in the PS of bull calves.

Elevated TIDA neuronal activity was maintained after 9 d of infusion of PRL, indicating that TIDA neurons in bulls do not become refractory to PRL-induced stimulation for at least 9 d.

There was no effect of haloperidol on synthesis or storage of 5HT in PS indicating that haloperidol does not

alter the activity of 5-hydroxytryptaminergic neurons in this brain region.

In summary, greatest concentrations of DA were localized in the PS and procedures that increased serum concentrations of PRL increased accumulation of DOPA in PS of bull calves. Therefore, TIDA neurons in the bovine hypothalamus most likely terminate in the PS and TIDA neurons in cattle are responsive to the feedback actions of PRL.

Chapter 3

Response of Tuberoinfundibular and 5-Hydroxytryptaminergic Neurons and Lactotropes to Photoperiod in Holstein Bull Calves

Introduction

Compared with exposure to 8L:16D, exposure to 16L:8D increased serum concentrations of PRL two- to eight-fold in cattle (Bourne and Tucker, 1975; Leining et al., 1979; Stanisiewski et al., 1984b, 1987b). However, the mechanism whereby photoperiod influences concentrations of PRL in cattle is unknown.

Several hypothalamic neurotransmitters, including DA and 5HT influence secretion of PRL (Jacobowitz, 1988). As described in the review of literature, DA from TIDA neurons tonically inhibits secretion of PRL. Conversely, 5HT stimulates secretion of PRL but not by direct action on the pituitary gland. 5HT may inhibit TIDA neurons (Nishihara et al., 1986; Nishihara and Kimura, 1987) or stimulate a PRL-releasing factor (Clemens et al., 1978) which increases secretion of PRL. Potentially, photoperiod-induced alterations in activity of TIDA and(or) 5-hydroxytryptaminergic neurons may be involved in photoperiod-induced changes in serum concentrations of PRL. Therefore, an objective of the current study was to determine if exposure to photoperiods that alter serum PRL alters estimates of activity of TIDA and(or) 5-hydroxytryptaminergic neurons in cattle.

In addition, photoperiod may induce change in secretion of PRL by causing changes in the lactotropes within the anterior pituitary gland. For example, following lesions

of hypothalamic input to the anterior pituitary gland, there is a gradual increase in the percentage of lactotropes in the pituitary gland (Phelps and Hymer, 1986). In addition, bromocriptine, a DA agonist, reduced the number of PRL secreting cells in vitro (Takahasi and Kawashima, 1987). Steger et al. (1982) reported that after 20 wk of exposure to 5L:19D, secretion of PRL increased without a change in concentrations of DA, suggesting a decreased sensitivity of the lactotrope to DA. Thus, photoperiod may influence the number and(or) secretory capacity of the lactotrope or change the sensitivity of the lactotrope to hypothalamic factors, which in turn results in photoperiod-induced alterations in serum concentration of PRL. Therefore, a second objective of the current study was to determine the effects of photoperiod on number and secretory capacity of the lactotrope and sensitivity of the pituitary gland to DA and TRH.

Materials and Methods

Sixteen prepubertal Holstein bull calves (approximately 8 wk of age) were assigned randomly to photoperiod treatments of 8L:16D or 16L:8D. Bulls were housed in individual stalls in light- and temperature- (20 ± 2°C) controlled chambers (four bulls/chamber). Cool-white fluorescent tubes at an average intensity of 400 lux

(measured 1 M from the floor) were utilized as the source of light. All bulls were exposed to 8L:16D for 6.5 wk. Lights came on at 0900 h and were turned off at 1700 h. Following this preliminary period, one-half of the bulls (n=8) were exposed to 16L:8D (lights on at 0430 h and off at 2030 h) and one-half of the bulls (n=8) remained on 8L:16D (lights on at 0430 h and off at 1230 h) for an additional 4 wk. Bulls were fed and given water as described in Chapter 2.

Two d before the switch in photoperiod, each bull was fitted with a polyvinyl cannula in a jugular vein. The next day blood was collected and discarded every 10 min from 0900 to 1050 h to accustom the bulls to sampling procedures. Beginning at 1100 h, blood samples were collected every 30 min for 6 h. Cannulation and collection of blood were repeated on all bulls 4 wk after the switch in photoperiod. Additional blood samples were collected from each bull 15 min before and immediately before euthanasia. Blood samples were stored at 20°C for 4 to 6 h and then overnight at 4°C. Samples of blood were centrifuged, sera decanted and stored at -20°C until assayed for PRL as described in Chapter 2.

Following collection of the last blood sample, all bulls were euthanized with sodium pentobarbital (iv; 85 mg/kg body weight). Fifteen min before euthanasia (immediately following collection of the penultimate blood sample) all bulls were injected iv with NSD 1015 (25 mg/kg

body weight) as described in Chapter 2. Accumulation of DOPA and 5HTP was used as an index of TIDA and 5HT neuronal activity, respectively, as described in Chapter 2.

Following euthanasia, a block of hypothalamic tissue was removed from each calf, frozen on dry ice and the PS, SCN and MBH were dissected as described in Chapter 2. In addition, the arcuate nucleus was dissected with a 1000 μ punch on each side of the third ventricle just anterior to the PS as shown in slice 1 (Figure 1C). Concentrations of DOPA, DA, 5HTP, 5HT and protein in the SCN, MBH, arcuate nucleus and in each slice of the PS were determined as described in Chapter 2. The pituitary gland was removed and transported to the laboratory on ice. Within 20 to 30 min of euthanasia, connective tissue and the posterior pituitary gland were removed and the anterior pituitary gland was weighed and bisected.

Cell dispersion and reverse hemolytic plaque assay. One-half of the anterior pituitary gland was stored at room temperature in Delbecco's modified Eagles medium (DMEM; Gibco Chemical Co., Grand Island, NY) with .1% BSA for determination of number and secretory capacity of individual lactotropes. Dispersed anterior pituitary cells were prepared by a modification of the method of Padmanabhan et al. (1978). Briefly, one-half of anterior pituitary gland from each bull was sliced (≈1 mm in thickness) in a Stadie-Riggs tissue slicer, then diced with

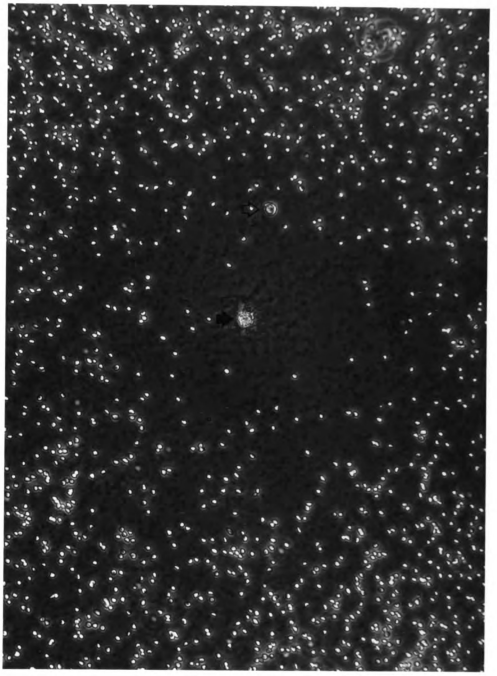
a scalpel blade (≈1 mm^3) and the resulting pieces washed four to five times in DMEM. Diced pieces were placed in a 125 ml flask with a stir bar with 30 ml of .10% collagenase (type 1; 150 U/mg; 37°C; Sigma) in DMEM. The stir bar was spun for 45 to 50 min causing dispersion of the pituitary pieces. Cells were then washed three times in DMEM. Cell clumps were dispersed further by stirring in .008% viokase (37°C; Gibco) in DMEM for 20 min. Cells were washed four times and resuspended in 2 ml of DMEM-.1% BSA. Cells were counted in a hemocytometer and between .5 x 10^5 and 10 x 10^5 cells/ml were used in the reverse hemolytic plaque assay according to a modification of the method of Smith et al. (1986) as described below.

One ml of dispersed anterior pituitary cells in DMEM-.1% BSA was mixed with an equal volume of ovine red blood cells (oRBC; Colorado Serum Co., Denver, CO) linked to staphylococcal protein-A (Sigma). Coupling of protein A to oRBC was prepared 1 d before use as described by Smith et al. (1986). The oRBC-pituitary cell mixture was loaded into Cunningham chambers (Cunningham and Szenberg, 1968), coated with poly-L-lysine (380,000 MW, Sigma; .25 mg/ml distilled water) as a monolayer. Eight to ten slides were prepared per animal. Slides were then incubated (37°C; 95% O_2:5% CO_2 at 100% humidity) for 45 min to permit attachment of oRBC-pituitary cells to the slide. Unattached cells were rinsed off with DMEM-.1% BSA. Antibody to bovine PRL produced in rabbits (diluted 1:40 in DMEM-.1% BSA; specific

binding of PRL antibody was 50% at a dilution of 1:20,000 in a double antibody radioimmunoassay) was added to each chamber. Slides were incubated for an additional 3 h. Antibody was rinsed off with DMEM-.1% BSA and guinea pig complement (diluted 1:40 in DMEM-.1% BSA; Gibco) was added to each chamber and reincubated for 30 min. Excess complement was rinsed out and cells fixed with glutaraldehyde [2% in Sorenson's phosphate buffer (70% .1M Na_2HPO_4 and 30% .1M KH_2PO_4)]. Slides were stored in Sorenson's buffer until analyzed.

A photograph of a representative plaque is shown in Figure 4. Area of each plaque was determined by centering the plaque in a microscopic field and selecting the ring of best fit on a calibrated concentric ring reticle. To identify non-plaque forming cells, cells were stained with toluidine blue (.05 g/100 ml borate buffer) and the number of plaque forming cells as a percentage of total pituitary cells was calculated. A minimum of three slides and 200 cells per bull calf were analyzed. Plaques were formed from lactotropes in 12 out of the 16 bulls (7 from 8L:16D and 5 from 16L:8D). No plaques were formed in four bulls due to technical problems. For example, in one bull no anterior pituitary cells were harvested and in three bulls the oRBC dried out, preventing formation of plaques. Only slides that had plaques were analyzed. Although plaques were formed on slides from 12 of the 16 bulls used in the

Figure 4. Photomicrograph of a plaque (zone of hemolysis) surrounding a PRL-secreting cell (solid arrow; ➧) viewed with phase contrast optics (magnified 400X). Non-plaque forming cell indicated by open arrow (⇨). RBC membrane ghosts can be seen in the plaque.



study, the number of plaques formed varied among bulls and among slides within a given bull. The biggest technical problem with the assay was maintaining hydration of the oRBC within the chamber. Once a chamber or part of a chamber dries out, no plaques are formed. Maintaining damp laboratory tissue around each slide and resoaking the tissue following each incubation reduced the problem of dehydration.

Pituitary explants. Explants were prepared by a modification of the method of Convey et al. (1973). Briefly, one-half of the anterior pituitary gland was sliced and diced as described previously and the resulting pieces washed four to five times in medium 199 (Sigma) with 25 mM HEPES (N-2-hydroxyethyl piperazine-N'-2-ethanesulfonic acid) and .244% sodium bicarbonate (M199). Two to three pieces were then placed into 12 x 75 mm test tubes containing 2 ml of M199. Tubes were incubated in a Dubnoff (Precision Scientific Co., Chicago, IL) metabolic shaker (37°C; 95% O_2:5% CO_2). Medium was replaced with 2 ml of M199 every 15 min for 1 h followed by a 2 h pretreatment incubation period. Medium was then harvested for determination of pretreatment concentrations of PRL. Two ml of M199 were then added to each tube. Explants were treated with 0, 10^{-6}, 10^{-8} or 10^{-10} M DA (in M199 with .01% ascorbic acid) or 10^{-7}, 10^{-9} or 10^{-11} M TRH (in M199) and incubated for an additional 2 h. There were two tubes/treatment per bull. Media were harvested for

determination of PRL. Explants were dried and weighed and data expressed as ng PRL/mg dry tissue.

Statistical analysis. DOPA, DA, 5HTP, 5HT in PS, SCN, MBH and arcuate nucleus and area of plaques and percentage of lactotropes data were analyzed by analysis of variance (Gill, 1978). PRL data (serum and media) were analyzed by split-plot analysis of variance with repeated measurement (Gill and Hafs, 1971). In addition, regression curves (linear and quadratic) were computed (Gill, 1978) to test response of PRL to increasing concentrations of DA and TRH.

Results

At the end of 6.5 wk of exposure to 8L:16D, concentrations of PRL were similar in both groups of calves, averaging 5.8 and 4.9 ng/ml of serum (Figure 5). After 4 wk, bulls exposed to 16L:8D had greater serum concentrations of PRL than bulls that remained on 8L:16D (35.8 vs 7.5 ng/ml; $P < .05$). Injection of NSD 1015 increased ($P < .01$) serum contractions of PRL within 15 min to 191.1 ± 35.5 from 35.8 ng/ml in bulls exposed to 16L:8D and to 43.0 ± 22.7 from 7.5 ng/ml in bulls exposed to 8L:16D.

Concentrations of DOPA, DA, 5HTP and 5HT, averaged across all slices of PS, are summarized in Table 10. Concentrations of DOPA were 14.7% greater than in bulls

Figure 5. Concentrations of prolactin in serum of prepubertal bull calves exposed for 6.5 wk to 8L:16D (solid symbols), then switched to 16L:8D (●) or maintained on 8L:16D (0) for an additional 4 wk. Pooled SE = 1.0 ng/ml.

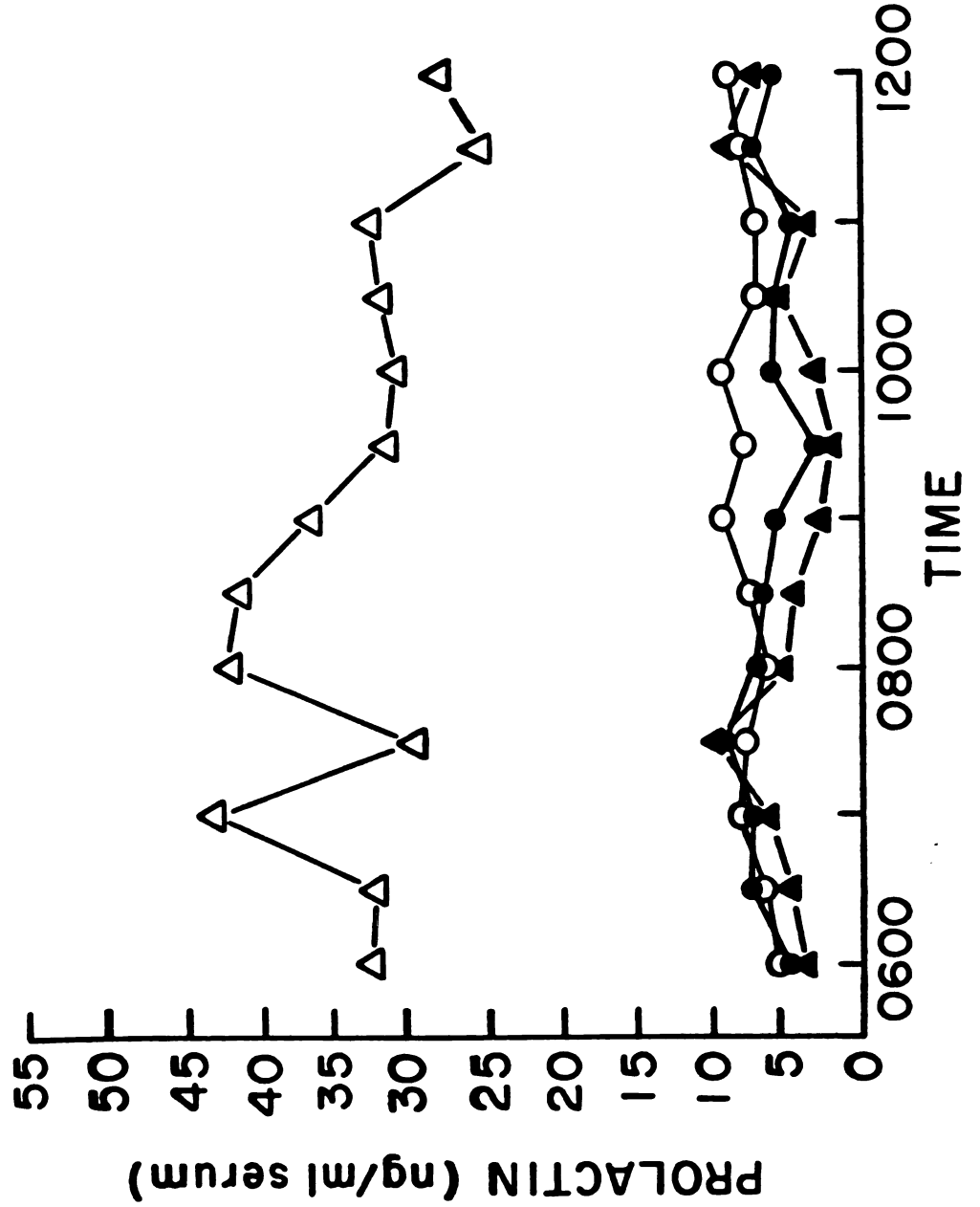

PROLACTIN (ng/ml serum)
55
50
45
40
35
30
25
20
15
10
5
0
0600
0800
1000
1200
TIME

Table 10.

Effects of photoperiod on accumulation of DOPA and 5HTP in the pituitary stalk of bull calves[a].

	8L:16D[b]	16L:8D[b]	SE[c]
DOPA	2.99	3.43	.21
DA	17.69	16.04	1.29
DOPA/DA	.17	.21[d]	.02
5HTP	1.26	1.08	.10
5HT	16.97	14.46	1.33
5HTP/5HT	.07	.07	.01

[a]Values represent the means of six bull calves for dihydroxyphenylalanine (DOPA; pg/μg protein), dopamine (DA; pg/μg protein), 5-hydroxytryptophan (5HTP; pg/μg protein) and 5-hydroxytryptamine (5HT; pg/μg protein).

[b]Within treatment values are averages of all sections of pituitary stalk.

[c]Pooled standard error.

[d]Means from 16L:8D differ from means of 8L:16D ($P < .10$).

exposed to 16L:8D than in bulls given 8L:16D. However, this difference was not significant. Photoperiod did not affect DA in PS. Exposure to 16L:8D tended ($P < .10$) to increase the ratio of DOPA to DA compared with exposure to 8L:16D. There was no effect of photoperiod on concentrations of 5HT or its precursor 5HTP in PS.

Concentrations of DOPA, DA, 5HTP and 5HT in the arcuate nucleus, MBH and SCN are summarized in Table 11. Concentrations of DOPA and DA in the MBH were greater in bulls exposed to 16L:8D than values in bulls subjected to 8L:16D. However, the ratio of DOPA to DA was not different between photoperiods. Photoperiod did not affect concentrations of DOPA or DA in the arcuate nucleus or SCN. Relative to 16L:8D, exposure to 8L:16D increased the concentrations of 5HTP and the ratio of 5HTP to 5HT but had no affect on 5HT in the arcuate nucleus. Concentrations of 5HT in the MBH tended to be greater ($P < .10$) than in bulls exposed to 16L:8D compared with 8L:16D. Photoperiod did not affect 5HTP or ratios of 5HTP to 5HT in the MBH nor were 5HTP, 5HT or ratios of 5HTP to 5HT affected.

Anterior pituitary glands from bulls exposed to 16L:8D were heavier ($P < .05$) than those from bulls given 8L:16D (595.7 ± 31.4 vs 485.2 ± 31.4 mg). In addition, the number of lactotropes as a percentage of total number of secretory cells of the anterior pituitary gland increased in bulls exposed to 16L:8D compared with bulls on 8L:16D (Table 10). Plaques formed around lactotropes from bulls exposed to

Table 11.

Effects of photoperiod on accumulation of DOPA and 5HTP in brain regions of bull calves[a].

	DOPA	DA	DOPA/DA	5HTP	5HT	5HTP/5HT
Arcuate Nucleus						
8L:16D	.90	1.48	.61	.37	3.76	.10
16L:8D	.94	1.72	.55	.28[c]	3.49	.08[c]
SE[b]	.16	.20	.07	.04	.31	.01
Mediobasal hypothalamus						
8L:16D	.28	1.06	.26	.23	4.04	.06
16L:8D	.66[d]	2.25[d]	.29	.27	5.68[c]	.05
SE[b]	.04	.28	.09	.05	.56	.01
Suprachiasmatic nucleus						
8L:16D	.16	1.06	.15	.41	1.98	.21
16L:8D	.16	.99	.16	.42	2.11	.20
SE[b]	.01	.10	.06	.03	.06	.02

[a]Values represent the means of six bull calves for dihydroxyphenylalanine (DOPA; pg/μ protein), dopamine (DA; pg/μ protein), 5-hydroxytryptophan (5HTP; pg/μg protein) and 5-hydroxytryptamine (5HT; pg/μg protein).

[b]Pooled standard error

[c]Means from 16L:8D differ from means of 8L:16D ($P < .10$).

[d]Means from 16L:8D differ from means of 8L:16D ($P < .05$).

16L:8D had 33% larger diameter and 70% larger area than bulls given 8L:16D (Table 12); indicating that photoperiods of 16L:8D increased the secretory capacity of lactotropes and increased the number of lactotropes.

Across all samples from the 2 h pretreatment incubation, pituitary gland explants from bulls exposed to 16L:8D secreted greater ($P < .05$) quantities of PRL into the media than explants from bulls exposed to 8L:16D (1232.1 ± 58 vs 786 ± 38 ng/mg dry tissue). However, compared with pretreatment, concentrations of PRL in the media were not changed following a 2 h incubation with DA or TRH in bulls exposed to 8 or 16 h of light (Table 13). In addition, slopes of response to increasing doses of DA or TRH were not different from zero ($P > .10$) in either group of bulls (data not shown). Thus, explants of anterior pituitary gland from bulls given 16L:8D secreted more PRL but explants from both groups of bulls were unresponsive to increasing doses of DA or TRH.

Discussion

Similar to previous reports (Bourne and Tucker, 1975; Leining et al., 1979; Stanisiewski et al., 1984b, 1987b), in the present study prepubertal Holstein bulls exposed to photoperiods of 16L:8D had greater serum concentrations of PRL than bulls exposed to 8L:16D. Since exposure to 16L:8D increased serum PRL, the objectives of the current study

Table 12.

Analysis of plaques formed from lactotropes in the reverse hemolytic plaque assay in anterior pituitary glands from bull calves exposed to photoperiod[a].

	8L:16D	16L:8D
Animals[b]	7	5
Diameter, mm	.104 ± .01	.139 ± .01[d]
Area, mm^2	.0093 ± .002	.0158 ± .002[d]
Lactotropes[c], %	11.2 ± 2.2	17.1 ± 2.6[e]

[a]Six to 10 slides were analyzed per bull calf.

[b]Only bull calves that produced plaques were included in the analysis.

[c]Number of lactotropes as a percentage of total number of pituitary cells. A minimum of three different slides and 200 cells were analyzed per bull calf.

[d]Values of 16L:8D are different from 8L:16D ($P < .05$).

[e]Values of 16L:8D are different from 8L:16D ($P < .10$).

Table 13.

Release of PRL from pituitary gland explants from bull calves exposed to 8L:16D or 16L:8D[a].

	8L:16D		16L:8D	
dose	Pretreatment[b]	Posttreatment	Pretreatment[b]	Posttreatment
0	840 ± 146	714 ± 98	1292 ± 222	1300 ± 256
DA 10^{-6}	834 ± 98	786 ± 82	1198 ± 180	1152 ± 344
10^{-8}	790 ± 114	722 ± 74	1132 ± 170	1544 ± 272
10^{-10}	816 ± 144	896 ± 142	1602 ± 268	1658 ± 322
TRH 10^{-7}	816 ± 110	904 ± 124	1222 ± 234	1186 ± 254
10^{-9}	676 ± 122	698 ± 108	1472 ± 312	1284 ± 276
10^{-11}	722 ± 60	720 ± 114	1326 ± 224	1264 ± 258

[a]Values represent the means (± SE) from eight bull calves. Data are expressed as ng prolactin/mg dry tissue. Explants were incubated for 2 h before treatment (pretreatment) and media collected and explants were then incubated for an additional 2 h (posttreatment) with dopamine (DA) at 0, 10^{-6}, 10^{-8} or 10^{-10} M or thyrotropin-releasing hormone (TRH) at 10^{-7}, 10^{-9} or 10^{-11} M.

[b]Overall pretreatment means from 16L:8D differ from 8L:16D ($P < .05$).

were to determine if photoperiods that alter PRL change: 1) activity of TIDA and 5-hydroxytryptaminergic neurons, 2) secretory capacity and number of lactotropes and 3) sensitivity of the anterior pituitary gland to DA and TRH.

Photoperiod-induced increments in serum concentrations of PRL begin to wane after exposure to 16L:8D for 12 to 16 wk in sheep and cattle (Almeida and Lincoln, 1984; Stanisiewski et al., 1987b). Thus, in terms of PRL secretion, sheep and cattle eventually become refractory to photoperiodic stimuli. In the present study, bulls exposed to 16L:8D for 4 wk tended to have greater activity of TIDA neurons (based on increased ratio of DOPA to DA in PS) than bulls exposed to 8L:16D. This increased activity of TIDA neurons may act to reduce secretion of PRL and may reflect the onset of refractoriness to photoperiodic stimulation of PRL.

The photoperiodic-induced increases in the ratio of DOPA to DA is most likely mediated by photoperiod-induced increases in concentrations of PRL. Indeed, as demonstrated in Chapter 2, elevated concentrations of PRL feed back to stimulate activity of TIDA neurons to reduce further secretion of PRL and the neurons remain responsive to PRL for at least 9 d in cattle (Experiment 2 and 3, Chapter 2).

Photoperiod-induced activation of TIDA neurons via increased serum PRL in the present study is in agreement with the results in Chapter 2. However, because DA from

TIDA neurons tonically inhibits PRL (Ben-Jonathan, 1985) it was originally postulated that exposure to 16L:8D would reduce activity of TIDA neurons, which in turn could permit serum concentrations of PRL to increase. The data in the current study do not preclude a role for TIDA neurons in the initiation of photoperiod-induced stimulation of serum PRL. In the present study, estimates of TIDA activity were made at a single point in time (4 wk) relative to the start of treatment. Measuring accumulation of DOPA in PS at several points in time in the first 4 wk relative to the beginning of photoperiod treatment should reflect the ontogeny of photoperiod-induced changes in TIDA neurons.

In bulls exposed to 16L:8D, concentrations of DOPA and DA were increased in the MBH but not in the arcuate nucleus or the SCN. However, photoperiod did not affect the ratio of DOPA to DA in the MBH, suggesting that although concentrations of DOPA and DA were greater in bulls exposed to 16L:8D there were no photoperiod-induced changes in activity of neurons in this region.

Perikarya of TIDA neurons are located in the arcuate nucleus (Ben-Jonathan, 1985; Albanese et al., 1986). 5HT can inhibit activity of TIDA neurons through its action on the arcuate nucleus (Nishihara et al., 1986; Nishihara and Kimura, 1987). In the current study, exposure to 16L:8D reduced synthesis of 5HT at the arcuate nucleus indicating reduced 5HT activity. Perhaps, photoperiod-induced

reductions in 5-hydroxytryptaminergic activity at the arcuate nucleus and thus, removal of its inhibitory action on TIDA neurons, is involved in the mechanism whereby 16L:8D increased activity of TIDA neurons in the current study.

The reverse hemolytic plaque assay allows microscopic visualization of hormone release at the level of a single cell (Frawley et al., 1986). The area of the plaque that develops around the individual cell provides an index of the relative amount of hormone released (Neill and Frawley, 1983; Frawley et al., 1985). That is, the larger the area of the plaque, the greater the secretion of hormone from that cell. In the current study, the reverse hemolytic plaque assay was used to determine secretory capacity and relative number of lactotropes from bovine anterior pituitary glands from prepubertal Holstein bulls exposed to long- or short-day photoperiods.

Individual lactotropes from bulls exposed to 16L:8D produced larger plaques and therefore, secreted greater quantities of PRL compared with bulls exposed to 8L:16D. In addition, exposure to 16L:8D increased the percentage of anterior pituitary cells that secrete PRL. The origin of these additional lactotropes is unknown. The increase may represent a recruitment of sommatomammotropes to secrete PRL. Treatment with estradiol causes a shift in cell types from GH-secreting to PRL-secreting cells in rats (Boockfor et al., 1986). Photoperiod may induce a similar shift in

cell type in cattle. In addition, since exposure to 16L:8D increased weight of pituitary glands, photoperiod may also increase hyperplasia of the lactotrope which would account for the increase in number of PRL-secreting cells. Indeed, following hypothalamic lesions in rats there is a gradual increase in the number of lactotropes (Phelps and Hymer, 1986) and treatment with bromocriptine reduced number of lactotropes (Takahasi and Kawashima, 1987).

The effect of photoperiod on PRL is a sluggish response. For example, following a switch from short- to long-day photoperiods, serum concentrations of PRL do not increase significantly for at least 4 d (Petitclerc et al., 1989) and do not reach a maximum for several weeks (Leining et al., 1979; Stanisiewski et al., 1984b). The sluggishness of the response of PRL to 16L:8D may be accounted for in the time required for photoperiod-induced increases in recruitment of or hyperplasia of lactotropes.

Pituitary explants from bulls exposed to 16L:8D secreted more PRL into the media. Unexpectedly, neither DA nor TRH affected release of PRL into the media. Using a similar incubation schedule, Stanisiewski et al. (1984c) reported a significant increase and decrease in secretion of PRL in bovine pituitary explants treated with TRH and DA, respectively. The reason for the failure to observe an effect of TRH or DA in the explants of the current study was unknown. However, Convey et al. (1973) using a similar

incubation schedule also failed to observe an effect of TRH on pituitary gland explants. Since TRH and DA were ineffective, it was not possible to determine a change in responsiveness of the pituitary gland to these hypothalamic factors. However, the increased serum concentrations of PRL in all bulls following injection of NSD 1015 [which blocks secretion of DA (Demarest et al., 1979) and reduces concentrations of DA in hypophyseal portal blood (Reymond and Porter, 1982)] indicate that secretion of PRL in bulls exposed to short- or long-day photoperiods are responsive to the inhibitory effects of DA.

In conclusion, exposure to long-day photoperiods of 16L:8D increased serum concentrations of PRL relative to exposure to short days. The increase in serum PRL may be mediated in part by an increased number of lactotropes and increased secretory capacity of the lactotropes. In addition, after 4 wk of exposure to long days, the elevated concentrations of PRL stimulated activity of TIDA neurons, which may indicate the start of photorefractoriness. This process may involve a change in 5HT input to the arcuate nucleus.

SUMMARY AND CONCLUSIONS

The objectives of this dissertation were to examine the effects of photoperiod on serum PRL, activity of TIDA and 5HT neurons in the hypothalamus and the secretory capacity and number of lactotropes in the anterior pituitary gland of prepubertal Holstein bull calves. Before the objectives could be addressed, validation of the methods was required. Accordingly, several studies were conducted to examine the effects of euthanasia with sodium pentobarbital on changes in concentrations of DOPAC as an estimate of activity of TIDA neurons in rats and the effects of elevated serum concentrations of PRL on concentrations of DOPAC and accumulation of DOPA as estimates of activity of dopaminergic neurons in the PS of prepubertal Holstein bull calves.

TIDA activity was increased in rats euthanized with sodium pentobarbital compared with decapitated rats. However, stress-induced decreases in activity of TIDA neurons were not masked in rats euthanized with sodium pentobarbital. Therefore, it was decided to test whether similar neurochemical measurements made in cattle euthanized with sodium pentobarbital were related to activity of TIDA neurons.

Concentrations of DA and DOPAC were greatest in the PS compared with MBH and SCN in Holstein bull calves indicating that terminals of TIDA neurons may be located in the PS in cattle compared with the MBH in rats. Therefore, dopaminergic activity in the PS was the focus for subsequent studies.

Injections of haloperidol, a DA antagonist, every 6 h for 24 h increased serum concentrations of PRL 6-fold compared with uninjected controls. Elevated serum PRL was maintained for 25 h. After 25 h of haloperidol-induced PRL or after 25 h of infusion of PRL, elevated serum concentrations of PRL increased concentrations of DOPA 60 to 88% and increased the ratio of DOPA to DA 120 to 150% in PS of Holstein bull calves relative to controls. Thus, greatest concentrations of DA are localized in PS and elevated serum concentrations of PRL increased accumulation of DOPA in PS of bull calves. Therefore, TIDA neurons in the bovine hypothalamus most likely terminate in the PS. In addition, TIDA neurons are responsive to feed back actions of elevated serum concentrations of PRL in cattle. For example, after 9 d of infusion of PRL based on increased accumulation of DOPA, activity of TIDA neurons was increased and therefore, TIDA neurons in bulls remain responsive to elevated serum concentrations of PRL after 9 d.

The conclusion drawn in these experiments that TIDA neurons most likely terminate in the PS was based on

quantity of and response of concentrations of DA. However, this conclusion has not yet been confirmed histologically. Future investigations should examine the anatomy of the bovine hypothalamus and identify histologically the location of the perikarya of TIDA neurons (arcuate nucleus) as well as the terminals of these neurons. In addition, the location of the MBH and SCN should be identified histologically in the bovine hypothalamus.

Relative to exposure to 8L:16D, exposure to 16L:8D for 4 wk increased serum concentrations of PRL approximately 5-fold in Holstein bull calves. Associated with the increased serum PRL was increased release of PRL into the media from pituitary explants from bulls exposed to 16L:8D. Individual lactotropes from bulls exposed to 16L:8D produced larger plaques and therefore, secreted greater quantities of PRL compared with bulls exposed to 8L:16D. Exposure to 16L:8D increased the percentage of anterior pituitary cells that secrete PRL indicating that bulls exposed to long days had increased number of lactotropes.

Exposure to 16L:8D tended to increase the ratio of DOPA to DA in PS relative to exposure to 8L:16D. Therefore, as demonstrated with procedures that elevate serum concentrations of PRL (i.e., haloperidol; infusion of PRL) photoperiod-induced elevated serum PRL can feed back to stimulate activity of TIDA neurons. Photoperiod did not affect accumulation of DOPA in MBH, SCN or arcuate nucleus

nor did photoperiod alter accumulation of 5HTP in PS, MBH or SCN. However, exposure to 16L:8D reduced accumulation of 5HTP in the arcuate nucleus compared with 8L:16D. The increased activity of TIDA neurons after 4 wk of exposure to 16L:8D may indicate the start of photorefractoriness of PRL secretion. This process may involve a change in 5HT input to the arcuate nucleus.

Given that DA tonically inhibits secretion of PRL, the results of the present study were unexpected. It was originally postulated that exposure to photoperiods of 16L:8D would decrease activity of TIDA neurons which would allow serum concentrations of PRL to increase. However, the results of the present study do not preclude a role of TIDA neurons in the onset of photoperiod-induced concentrations of PRL. Measuring activity of TIDA neurons at several points in time relative to the beginning of photoperiod treatment should reflect the ontogeny of photoperiod-induced changes in these neurons. I believe a study of ontogeny is a next logical experiment in determining the role of TIDA neurons in photoperiod-induced changes in serum concentrations of PRL.

In conclusion, exposure to long-day photoperiods of 16L:8D increased serum concentrations of PRL relative to exposure to short days. The increase in serum PRL may be mediated in part by increased number of lactotropes and increased secretory capacity of the lactotropes. In addition, after 4 wk of exposure to long days, the elevated

concentrations of PRL stimulated activity of TIDA neurons, which may indicate the start of photorefractoriness. This process may involve a change in 5HT input to the arcuate nucleus. Ontogeny studies are required to more completely understand the role of TIDA and(or) 5-hydroxytryptaminergic neurons in photoperiod-induced increases in serum concentrations of PRL in cattle.

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