Effects of dopamine on prolactin secretion and cyclic AMP accumulation in the rat anterior pituitary gland

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The effects of dopamine on pituitary prolactin secretion and pituitary cyclic AMP accumulation were studied by using anterior pituitary glands from adult female rats, incubated *in vitro*. During 2 h incubations, significant inhibition of prolactin secretion was achieved at concentrations between 1 and 10 nM-dopamine. However, 0.1–1 μ M-dopamine was required before a significant decrease in pituitary cyclic AMP content was observed. In the presence of 1 μ M-dopamine, pituitary cyclic AMP content decreased rapidly to reach about 75% of the control value within 20 min and there was no further decrease for at least 2 h. Incubation with the phosphodiesterase inhibitors theophylline (8 mM) or isobutylmethylxanthine (2 mM) increased pituitary cyclic AMP concentrations 3- and 6-fold respectively. Dopamine (1 μ M) had no effect on the cyclic AMP accumulation measured in the presence of theophylline, but inhibited the isobutylmethylxanthine-induced increase by 50%. The dopamine inhibition of prolactin secretion was not affected by either inhibitor. Two derivatives of cyclic AMP (dibutyryl cyclic AMP and 8-bromo cyclic AMP) were unable to block the dopamine (1 μ M) inhibition of prolactin secretion, although 8-bromo cyclic AMP (2 mM) significantly stimulated prolactin secretion and both compounds increased somatotropin (growth hormone) release. Cholera toxin (3 μ g/ml for 4 h) increased pituitary cyclic AMP concentrations 4–5-fold, but had no effect on prolactin secretion. The inhibition of prolactin secretion by dopamine was unaffected by cholera toxin, despite the fact that dopamine had no effect on the raised pituitary cyclic AMP concentration caused by this factor. Dopamine had no significant effect on either basal or stimulated somatotropin secretion under any of the conditions tested. We conclude that the inhibitory effects of dopamine on prolactin secretion are probably not mediated by lowering of cyclic AMP concentration, although modulation of the concentration of this nucleotide in some other circumstances may alter the secretion of the hormone.

The secretion of prolactin from the anterior pituitary gland appears to be controlled, to a large extent, by factors released from the hypothalamus into the hypophyseal portal system. In mammals this regulation appears to be predominantly inhibitory, and there is now considerable experimental evidence to suggest that dopamine is the most important physiological release-inhibiting factor involved (for reviews see MacLeod, 1976; Reichlin *et al.*, 1976; Vale *et al.*, 1977; Schally *et al.*, 1978). Prolactin-releasing factors have also been reported. In some systems thyroliberin (thyrotropin-releasing hormone) has been shown to stimulate prolactin secretion *in vitro* (MacLeod, 1976; Vale *et al.*, 1977); however, the physiological significance of these factors in the control of prolactin secretion *in*

vivo remains unclear. Substances with opiate-like activity may be involved in the stimulation of prolactin secretion (Enjalbert *et al.*, 1979; Gold *et al.*, 1979) through their effects on the release of hypothalamic regulatory factors for prolactin including dopamine (Van Loon *et al.*, 1980). It has been shown that opiates can antagonize the dopamine inhibition of prolactin secretion *in vitro* (Enjalbert *et al.*, 1979); thus these substances may also have direct actions at the pituitary level.

Dopamine-mediated inhibition of prolactin secretion is now considered to occur at least partly through a direct action of the catecholamine on the prolactin-secreting cells of the pituitary (MacLeod, 1976; Ben-Jonathan *et al.*, 1977). Specific dopamine receptors have been identified in pituitary tissue

(Caron *et al.*, 1978; Cronin *et al.*, 1978) but these binding sites have not yet been localized specifically to the prolactin-secreting cells. Also the mechanism of dopamine action, through which receptor binding leads to the inhibition of prolactin secretion, has to be explained.

It has been proposed that cyclic AMP may play a central role as a cellular mediator of the actions of certain hypothalamic regulatory hormones in the anterior pituitary gland (Labrie *et al.*, 1976, 1979a,b). According to this hypothesis, stimulation of prolactin secretion from the lactotrophs could occur via processes that act to increase cellular cyclic AMP concentration. Conversely, the dopamine-mediated inhibition of prolactin secretion could be explained by a mechanism that involved the lowering of cyclic AMP concentration in the prolactin-secreting cells.

The evidence that cyclic AMP is involved in the regulation of prolactin secretion is not entirely conclusive. Thyroliberin has been reported to increase prolactin secretion by stimulating cyclic AMP accumulation in cultured pituitary cells (Dannies *et al.*, 1976) and purified lactotrophs (Barnes *et al.*, 1978). Several groups have reported that butyryl derivatives of cyclic AMP stimulated prolactin secretion (Lemay & Labrie, 1972; Dannies *et al.*, 1976; Hill *et al.*, 1976), but this effect was not consistently observed (Hill *et al.*, 1976) and several alternative derivatives had little or no activity (Cehovic *et al.*, 1972, 1976). Dannies *et al.* (1976) have shown, by using cultured pituitary cells, that the effects of dibutyryl cyclic AMP on secretion could be reproduced by equivalent amounts of sodium butyrate. In general, the use of phosphodiesterase inhibitors has been shown to increase the rate of prolactin secretion (Parsons & Nicoll, 1970; Kimura *et al.*, 1976; Nakano *et al.*, 1976).

The biochemical mode of action of dopamine has not been investigated in much detail but its effect on prolactin secretion has been linked with a cyclic AMP-dependent mechanism. Dibutyryl cyclic AMP has been reported to block the inhibition of prolactin secretion by dopamine (Hill *et al.*, 1976); however, this effect was also observed with adenosine. In pituitary tissue dopamine was able to inhibit both the increase in prolactin secretion and cyclic AMP accumulation produced by theophylline (Kimura *et al.*, 1976). By using an enriched lactotroph preparation, Barnes *et al.* (1978) have shown that dopamine inhibited the increase in cyclic AMP induced by thyroliberin and Labrie *et al.* (1979b) have reported a rapid decrease in cyclic AMP accumulation in male rat pituitaries that was maximal 5 min after dopamine addition. In the present study inhibition of prolactin secretion by dopamine and its effects on cyclic AMP have been measured under a variety of conditions that were

designed to modulate pituitary cyclic AMP concentration *in vitro*. As far as is known the agents used (cyclic AMP derivatives, phosphodiesterase inhibitors and cholera toxin) do not exhibit specificity for particular pituitary cell types. Thus, gross changes in pituitary cyclic AMP concentration should be indicative of the changes occurring in the lactotrophs as well as the cells that secrete other hormones.

The aim of this work was to examine further the potential relationship between dopamine, cyclic AMP and prolactin secretion so that a biochemical mechanism for dopamine action could be established. A preliminary communication describing some aspects of this work has been published (Ray & Wallis, 1980).

Experimental

Materials

[2,8-³H]Adenosine 3':5'-cyclic phosphate (sp. radioactivity 30–50 Ci/mmol) was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. Dibutyryl cyclic AMP and 8-bromo cyclic AMP were from The Boehringer Corporation (London) Ltd., Lewes, E. Sussex, U.K. Isobutylmethylxanthine, theophylline and cholera toxin (from *Vibrio cholerae*) were obtained from the Sigma Chemical Co., Poole, Dorset, U.K. Rat prolactin (NIAMDD-Rat Prolactin-RP-1; 11 i.u./mg), rat somatotropin (growth hormone; NIAMDD-rat GH-B-1; 0.9 i.u./mg) and antisera for the radioimmunoassay of these hormones were gifts from Dr. A. Parlow and the Rat Pituitary Hormone Program of the National Institute of Arthritis, Metabolism and Digestive Diseases, NIH, Bethesda, MD, U.S.A.

Experimental incubations

Anterior pituitary glands were removed from 8–10-week-old virgin female Sprague–Dawley rats. These animals were obtained from the laboratory colony and were fed *ad lib*. Paired anterior hemipituitaries were preincubated at 37°C in air/CO₂ (19:1) for 60 min in Krebs–Ringer bicarbonate medium (0.5 ml/hemipituitary) containing 118 mM-NaCl, 4.7 mM-KCl, 2.5 mM-CaCl₂, 1.2 mM-MgSO₄, 25 mM-NaHCO₃, 1.2 mM-KH₂PO₄, 20 mg of Phenol Red/litre, 35 mg of penicillin G/litre and 100 mg of streptomycin sulphate/litre, giving a final pH of about 7.4. At 20 min intervals the glands were rinsed with fresh Krebs buffer to remove any erythrocytes and cell debris, which were discarded. After the final rinse at 60 min, 0.5 ml of Krebs buffer containing the drug (experimental) or solvent buffer (control) was added to the paired hemipituitary glands to initiate the experiment. For all experiments dopamine was protected from oxidation by the inclusion of 0.1 mM-

ascorbic acid and 1 mM-thiourea in the incubation buffer (Barber *et al.*, 1977). Unless stated otherwise the concentration of dopamine used was 1 μ M and the incubation period was 2 h. At the end of this period the media were carefully removed and quick-frozen in a solid-CO₂/ethanol mixture. Pituitary tissue was immediately homogenized in 1 ml of ice-cold trichloroacetic acid (5%, w/v) containing cyclic [2,8-³H]AMP (approx. 10000 d.p.m.) to monitor recovery and the homogenates were placed on ice or frozen. Incubations with paired hemipituitaries were run using at least three replicate points for each experimental condition.

Measurement of cyclic AMP

At the end of the experiment the homogenates were centrifuged and the supernatants were subjected to chromatography on Dowex 50 (X8; 100–200 mesh) (Kelly & Butcher, 1974). The cyclic AMP-containing fractions were eluted with water and concentrated by freeze-drying. Samples were finally redissolved in 1 ml of sodium acetate/acetic acid buffer (50 mM with respect to sodium acetate), pH 4.75, and cyclic AMP was determined by a radioimmunoassay procedure. Briefly, a diluted portion of each sample containing 50–500 fmol of cyclic AMP was acetylated (Brooker *et al.*, 1979) and then incubated with ¹²⁵I-labelled succinylated cyclic AMP (approx. 20000 d.p.m.; Steiner *et al.*, 1972) and antibody by the method of Brooker *et al.* (1979). After an overnight incubation at 4°C, free and bound cyclic AMP were separated by the addition of charcoal (Norit GSX) followed by centrifugation (approx. 2000 g for 20 min) at 0–4°C. A portion of each supernatant was then counted for gamma radiation to determine the amount of tracer that remained bound to the antibody. These values were then compared with a standard displacement curve (10–10000 fmol of cyclic AMP) to evaluate the cyclic AMP content of individual samples. A second portion of each sample was counted in scintillation fluid [toluene/Triton, 2:1 (v/v) containing 0.25% (w/v) 2,5-diphenyloxazole] to determine the recovery of cyclic [2,8-³H]AMP tracer. Material precipitated at the homogenization step with trichloroacetic acid was redissolved in 0.1 M-NaOH and protein was estimated by the method of Lowry *et al.* (1951). Corrected measurements of pituitary tissue cyclic AMP concentrations are expressed as pmol of cyclic AMP/mg of tissue protein.

Measurements of prolactin and somatotropin secretion

Media samples containing prolactin and somatotropin released during the experimental incubation period were diluted (usually 400–2000-fold) in a

buffer containing 50 mM-NaH₂PO₄, adjusted to pH 7.6 with NaOH, bovine serum albumin (0.5 mg/ml) and 0.6 mM-merthiolate. Radioimmunoassays for these hormones were performed by using the standards and antisera provided by Dr. A. Parlow and the National Institute of Arthritis, Metabolism and Digestive Diseases. The procedure adopted was that described in detail by Wallis *et al.* (1980). The antibody-hormone complex was precipitated with poly(ethylene glycol), as described by Desbuquois & Aurbach (1971). The amounts of hormone released were estimated from the standard curve(s) and expressed in terms of μ g of NIAMDD prolactin (or somatotropin) standard/mg of pituitary protein.

Expression of results

Values for pituitary cyclic AMP concentration and hormone released are expressed as means $\pm$ S.E.M. for the results obtained from replicate (*n*) experimental incubations. Control and experimental values for these measurements were compared for statistical differences by using a modification of Student's *t* test for 'paired comparisons' (Bailey, 1976).

Results

Dopamine dose-response relationships

The effects of dopamine concentration on prolactin secretion and pituitary cyclic AMP concentration are illustrated in Fig. 1. Values for the prolactin released in the presence of dopamine during a 2 h incubation are expressed as percentages of the paired controls. A significant ($P < 0.05$) inhibition of prolactin secretion was achieved at 10 nM-dopamine. Half-maximal inhibition of secretion was reached at approx. 14 nM-dopamine and at 1 μ M-dopamine the inhibition was almost maximal. A similar treatment of the data has been used to compare the cyclic AMP concentrations measured in the same paired hemipituitaries. The effect of dopamine concentration on the cyclic AMP concentration measured after 2 h is shown in Fig. 1(b). It can be seen that at this time only the highest concentration of dopamine tested, 1 μ M, had a significant effect ($P < 0.05$) on pituitary cyclic AMP content. Under these conditions cyclic AMP was decreased to about 80% of the concentration measured in the paired control hemipituitaries.

Time course for dopamine action

The effects of dopamine (1 μ M) on prolactin release (Fig. 2a) and pituitary cyclic AMP content (Fig. 2b) were measured at various times over the 2 h incubation period. In the absence of dopamine, prolactin secretion was essentially linear over the first hour. Between 1 and 2 h secretion appeared to

Fig. 1. Effects of dopamine concentration on pituitary prolactin secretion and cyclic AMP concentration

Paired hemipituitary glands from adult female rats were incubated for 2 h in the absence or presence of dopamine at the concentrations shown. At the end of the incubation period the media were removed and quick-frozen. The glands were homogenized immediately in ice-cold trichloroacetic acid (5%, w/v) to fix cellular cyclic AMP concentrations. ³H-labelled cyclic AMP was included as an internal marker for recovery during the subsequent fractionation. Prolactin in the incubation media and cyclic AMP in the cellular extracts were measured by the procedures described in the Experimental section. At each dopamine concentration tested, values for (a) prolactin secreted and (b) pituitary cyclic AMP content are expressed as percentages of the untreated paired control values. The values shown are the means for six determinations measured in two separate experiments. Vertical bars indicate $\pm$ S.E.M.

Fig. 2. Time courses for the effects of dopamine on pituitary prolactin secretion and cyclic AMP concentration

Paired anterior hemipituitary glands from female rats were incubated either in the absence (O) or presence (●) of 1 µM-dopamine. At the times indicated, incubations were terminated by removal of the media and the glands were homogenized immediately in ice-cold trichloroacetic acid (5%, w/v) to fix cellular cyclic AMP concentrations. ³H-labelled cyclic AMP was included as an internal marker for recovery during subsequent fractionation. Prolactin in the incubation media and cyclic AMP in the cellular extracts were measured by the procedures described in the Experimental section. At each time point the values given for (a) prolactin secreted and (b) pituitary cyclic AMP content are the means for at least six determinations, with vertical bars indicating $\pm$ S.E.M. Paired control and experimental values were compared for statistical differences by Student's *t* test. The probability values are: **P* < 0.05; ***P* < 0.002; ****P* < 0.001 (compared with appropriate controls).

fall off slightly in this experiment, but in most experiments a linear release was observed for periods of up to 4 h. In the presence of dopamine, secretion was significantly inhibited by 20 min and after 1 h medium concentrations of prolactin had only reached 56% of the control values. As there was no further increase in the prolactin measured in the medium between 1 and 2 h this suggests that secretion had been completely inhibited by this stage.

The cyclic AMP content of dopamine-treated pituitaries was apparently decreased at the earliest time tested, 5 min, but the effect was not statistically significant until 20 min. There was no further decrease throughout the two hour incubation period, with cyclic AMP concentrations remaining at approx. 75% of the control values.

Interestingly, during the period of preincubation after removal of the glands from the animals, pituitary cyclic AMP concentrations fell markedly. Typical values were 25 pmol/mg of protein immediately *post mortem* compared with about 8 pmol/mg for the experimental controls after 1 h. As control values did not vary significantly throughout the subsequent experimental incubation period this provides an indication that pituitary cyclic AMP concentrations had been effectively stabilized by the initial treatment.

Basal somatotropin secretion, which was measured in the same experiment, occurred at a linear rate throughout, and by the end of the 2 h period somatotropin released into the medium had reached $7.1 \pm 0.8 \mu\text{g}/\text{mg}$ of pituitary protein. The secretion of somatotropin was not significantly affected by the presence of dopamine ($1 \mu\text{M}$); thus these results demonstrate a selective action of the catecholamine on prolactin-secreting cells.

Effects of phosphodiesterase inhibitors

A potential relationship between cyclic AMP and prolactin secretion has been suggested by reports that phosphodiesterase inhibitors increase the rate of release of the hormone from pituitary tissue and cells (Kimura *et al.*, 1976; Nakano *et al.*, 1976). In the following experiments we have re-examined this relationship, and investigated the effects of dopamine on prolactin secretion and cyclic AMP in the presence of two such inhibitors, theophylline and isobutylmethylxanthine. The actions of these agents on somatotropin secretion were also measured.

The data in Table 1 summarize the results obtained from three experiments. In the presence of dopamine ($1 \mu\text{M}$) alone there was the expected inhibition of prolactin secretion, but in this series of experiments the effect on basal cyclic AMP was not significant. Theophylline (8 mM) appeared to increase secretion by 60–90% (though the control value was unusually low in this case). Cyclic AMP concentrations reached three times the untreated control values during a 2 h incubation with theophylline. Addition of dopamine completely blocked the apparent stimulatory effect of theophylline on prolactin secretion and in fact caused a significant inhibition of release. However, there was no significant effect of dopamine on the accumulation of cyclic AMP caused by theophylline. Somatotropin secretion was also increased in incubations that contained theophylline and the effect was maintained even when dopamine was included. This result indicates that the dopamine inhibition of theophylline-stimulated hormone secretion was specific for prolactin-secreting cells.

Isobutylmethylxanthine, an alternative phosphodiesterase inhibitor, produced an even greater effect on pituitary cyclic AMP accumulation. Cyclic AMP

Table 1. *Effects of theophylline and dopamine on prolactin and somatotropin secretion and pituitary cyclic AMP concentration*

Paired anterior hemipituitary glands from female rats were incubated for 2 h in Krebs–Ringer bicarbonate buffer, either alone or containing dopamine ($1 \mu\text{M}$), theophylline (8 mM) or a combination of these compounds. At the end of the incubation period the media were removed and frozen. The glands were homogenized immediately in ice-cold trichloroacetic acid to fix cellular cyclic AMP concentrations. Prolactin in the media and cyclic AMP in the pituitary extracts were measured subsequently as described in the Experimental section. Each value represents the mean $\pm$ S.E.M. for nine observations. Paired experimental and control values were compared for statistical differences by Student's *t* test. The probability values are: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Conditions	Hormone released ($\mu\text{g}/\text{mg}$ of protein)		Cyclic AMP (pmol/mg of protein)
	Prolactin	Somatotropin	
Control	9.1 ± 1.6	8.6 ± 0.9	8.0 ± 1.0
Dopamine ($1 \mu\text{M}$)	$4.5 \pm 0.8^*$	7.7 ± 1.2	7.2 ± 0.9
Control	6.3 ± 0.9	6.0 ± 0.4	7.6 ± 1.1
Theophylline (8 mM)	$10.6 \pm 0.7^*$	$11.6 \pm 1.2^{***}$	$21.9 \pm 2.2^{***}$
Control	8.1 ± 0.9	8.3 ± 1.0	7.0 ± 0.9
Dopamine ($1 \mu\text{M}$) + theophylline (8 mM)	$5.4 \pm 1.1^*$	$12.1 \pm 2.0^{**}$	$17.4 \pm 2.4^{***}$

concentrations in isobutylmethylxanthine-treated hemipituitaries were 6–7-fold higher than those measured in the untreated paired controls. Despite this marked effect on cyclic AMP there was no significant increase in prolactin secretion (Table 2). The inhibition by dopamine of prolactin secretion was not overcome by the presence of isobutylmethylxanthine. However, the accumulation of cyclic AMP caused by isobutylmethylxanthine was severely attenuated when incubations contained dopamine. An interesting finding was that the stimulation by isobutylmethylxanthine of somatotropin secretion was unaffected by the presence of dopamine, even though pituitary cyclic AMP concentrations were decreased by over 50%.

Effects of cyclic AMP derivatives

Derivatives of cyclic AMP have been found to

induce cyclic AMP-mediated actions when added exogenously to intact tissue or cells. We were therefore interested to determine what effects these agents would have on prolactin secretion and its inhibition by dopamine. In two experiments (summarized in Table 3) dibutyryl cyclic AMP (2 mM) had no effect on prolactin secretion and was unable to block the inhibitory effect of dopamine on the release of this hormone. Although dibutyryl cyclic AMP by itself had no apparent effect on somatotropin secretion, when the derivative was added in the presence of dopamine a significant stimulation of somatotropin secretion was observed.

Since some of the actions of dibutyryl cyclic AMP on prolactin secretion may result from the effects of metabolites derived from this compound rather than direct cyclic nucleotide-mediated events (Dannies *et al.*, 1976; Hill *et al.*, 1976), the activity of a second,

Table 2. *Effects of isobutylmethylxanthine and dopamine on prolactin and somatotropin secretion and pituitary cyclic AMP concentration*

Paired anterior hemipituitary glands from female rats were incubated for 2 h in Krebs–Ringer bicarbonate buffer, either alone or containing dopamine (1 μ M), isobutylmethylxanthine (2 mM) or a combination of these compounds. At the end of the incubation period the media were removed and frozen. The glands were homogenized immediately in ice-cold trichloroacetic acid to fix cellular cyclic AMP concentrations. Prolactin and somatotropin in the media, and cyclic AMP in the pituitary extracts, were measured as described in the Experimental section. Each value represents the mean $\pm$ S.E.M. for the number of observations given in parentheses. Paired experimental and control values were compared for statistical differences by Student's *t* test. Probability values are **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Conditions	Hormone released (μ g/mg of protein)		Cyclic AMP (pmol/mg of protein)
	Prolactin	Somatotropin	
Control	10.6 $\pm$ 1.6 (9)	6.5 $\pm$ 1.7 (6)	6.0 $\pm$ 0.7 (9)
Dopamine (1 μ M)	5.6 $\pm$ 0.7*	6.8 $\pm$ 1.9	5.7 $\pm$ 0.4
Control	11.0 $\pm$ 1.3 (9)	7.2 $\pm$ 2.7 (6)	6.1 $\pm$ 0.4 (9)
Isobutylmethylxanthine (2 mM)	13.5 $\pm$ 1.2	11.7 $\pm$ 1.7	41.8 $\pm$ 5.3***
Control	12.2 $\pm$ 1.0 (6)	3.8 $\pm$ 0.4 (3)	7.5 $\pm$ 0.8 (6)
Dopamine (1 μ M) + isobutylmethylxanthine (2 mM)	5.5 $\pm$ 0.5***	8.3 $\pm$ 0.7***	17.4 $\pm$ 1.2***

Table 3. *Effects of dibutyryl cyclic AMP and dopamine on prolactin and somatotropin secretion*

Paired anterior hemipituitary glands from female rats were incubated for 2 h in Krebs–Ringer bicarbonate buffer, either alone or containing dopamine (1 μ M) plus or minus dibutyryl cyclic AMP (2 mM). Prolactin and somatotropin in the media were measured as described in the Experimental section. Each value for the amount of hormone secreted is given as the mean $\pm$ S.E.M. for six observations. Paired experimental and control values were compared for statistical differences by Student's *t* test. Probability values are: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Conditions	Hormone released (μ g/mg of protein)	
	Prolactin	Somatotropin
Control	10.6 $\pm$ 1.4	6.7 $\pm$ 0.9
Dopamine (1 μ M)	5.2 $\pm$ 0.7***	7.4 $\pm$ 0.8
Control	10.7 $\pm$ 1.3	8.2 $\pm$ 1.6
Dibutyryl cyclic AMP (2 mM)	10.0 $\pm$ 0.6	9.3 $\pm$ 1.0
Control	9.8 $\pm$ 1.5	8.0 $\pm$ 1.6
Dibutyryl cyclic AMP (2 mM) + dopamine (1 μ M)	5.8 $\pm$ 1.2**	14.4 $\pm$ 1.2*

Table 4. *Effects of 8-bromo cyclic AMP and dopamine on prolactin and somatotropin secretion*

Paired anterior hemipituitary glands from female rats were incubated for 2 h in Krebs–Ringer bicarbonate buffer, either alone or containing dopamine (1 μ M) plus or minus 8-bromo cyclic AMP (2 mM). Prolactin and somatotropin in the media were measured as described in the Experimental section. Each value represents the mean $\pm$ S.E.M. for the number of observations given in parentheses. Paired experimental and control values were compared for statistical differences by Student's *t* test. Probability values are: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Conditions	Hormone released (μ g/mg of protein)	
	Prolactin	Somatotropin
Control	9.3 $\pm$ 1.3 (9)	6.9 $\pm$ 1.3 (9)
Dopamine (1 μ M)	4.1 $\pm$ 0.5*** (9)	6.3 $\pm$ 0.9 (9)
Control	9.0 $\pm$ 0.9 (9)	6.8 $\pm$ 0.9 (9)
8-Bromo cyclic AMP (2 mM)	11.6 $\pm$ 1.4* (9)	11.9 $\pm$ 1.6** (9)
Control	9.2 $\pm$ 1.4 (6)	6.4 $\pm$ 1.0 (6)
Dopamine (1 μ M) + 8-bromo cyclic AMP (2 mM)	4.0 $\pm$ 0.6*** (6)	10.4 $\pm$ 2.0* (6)

Table 5. *Effects of cholera toxin and dopamine on prolactin and somatotropin secretion and pituitary cyclic AMP accumulation*

Paired anterior hemipituitary glands from female rats were incubated for 4 h in Krebs–Ringer bicarbonate buffer either alone or in the presence of dopamine (1 μ M). Cholera toxin (3 μ g/ml) was also included in the incubation media as shown. Prolactin and somatotropin in the media and cyclic AMP in the glands were measured as described in the Experimental section. Each value represents the mean $\pm$ S.E.M. for at least 11 observations. Probability values (by Student's *t* test), compared with corresponding controls, are: **P* < 0.01; ***P* < 0.001; †*P* < 0.05.

Conditions	Cholera toxin	Hormone released (μ g/mg of protein)		Cyclic AMP (pmol/mg of protein)
		Prolactin	Somatotropin	
Control	—	8.4 $\pm$ 1.0	8.9 $\pm$ 1.1	5.5 $\pm$ 0.2
Dopamine (1 μ M)	—	2.7 $\pm$ 0.6**	9.9 $\pm$ 1.6	4.6 $\pm$ 0.2*
Control	+	9.5 $\pm$ 1.3	13.8 $\pm$ 1.8†	23.3 $\pm$ 2.7
Dopamine (1 μ M)	+	3.5 $\pm$ 0.4**	14.1 $\pm$ 1.9	20.9 $\pm$ 2.3

more stable cyclic AMP derivative was tested. Incubations made in the presence of 8-bromo cyclic AMP (2 mM) alone produced a small but significant increase in prolactin secretion when compared with controls (Table 4). However, once again the derivative was unable to block the inhibition of prolactin secretion that occurred when dopamine was included. Measurements of somatotropin secreted from the 8-bromo cyclic AMP-treated hemipituitaries were higher than the control values, both in the presence and absence of dopamine.

Effects of cholera toxin

The action of cholera toxin on a great variety of mammalian tissues is characterized by an essentially irreversible activation of adenylate cyclase leading eventually to raised intracellular concentrations of cyclic AMP (Gill, 1977). The effects of cholera toxin on pituitary cyclic AMP accumulation and the secretion of prolactin and somatotropin were measured both in the absence and presence of dopamine (Table 5). A 4 h incubation period was adopted to allow a substantial 4–5-fold increase in cyclic AMP accumulation. Under these conditions there was a significant 40–50% increase in somato-

tropin secretion but no effect on prolactin secretion was observed. In this experiment prolactin secretion was rather lower than usual, but secretion was approximately linear over the 4 h period both in the presence and absence of cholera toxin.

The dopamine inhibition of prolactin secretion was associated with a small but significant decrease in cyclic AMP accumulation. The inhibition of prolactin secretion was essentially unchanged in the presence of cholera toxin despite a 4–5-fold rise in pituitary cyclic accumulation. The toxin-induced increases in somatotropin secretion and cyclic AMP accumulation were not significantly affected by the inclusion of dopamine in the incubations.

Discussion

The initial purpose of the present study was to establish whether cyclic AMP is directly involved in the dopamine-mediated inhibition of prolactin secretion. Preliminary observations indicated that dopamine was able to lower the 'basal' cyclic AMP content of hemipituitaries incubated *in vitro* and that this change might be temporally related to a decrease in the rate of prolactin secretion. The fall in

basal pituitary cyclic AMP concentration after the 2h incubation with dopamine ($1\mu\text{M}$) was small and in two experiments (Tables 1 and 2) a significant difference from the control was not established. When the data of Figs. 1 and 2 and Tables 1, 2 and 5 were pooled, the mean values for the cyclic AMP concentrations in control and dopamine-treated glands were 7.2 ± 0.4 (S.E.M.; $n = 44$) and 6.3 ± 0.4 (S.E.M.; $n = 44$) pmol/mg of protein respectively. The difference between these values was highly significant ($P < 0.001$ by Student's t test), when paired values were compared.

Consideration of the dose-response curves for dopamine action, however, suggests that the two effects of this catecholamine might not be directly correlated. As shown in Fig. 1(a), a half-maximal inhibitory effect on prolactin secretion was achieved at approx. 10 nM -dopamine. This concentration is slightly lower than, but close to, the value of 35 nM reported by Caron *et al.* (1978) for rat pituitary cells in primary culture. Interestingly, we have found that a much higher concentration of dopamine was required to significantly inhibit cyclic AMP accumulation (Fig. 1b). At 100 nM -dopamine, a dose that produced an almost maximal inhibition of secretion, there was no apparent inhibition of cyclic AMP accumulation. This apparent difference in the dose-response relationships might be explained if the effects of dopamine on secretion and cyclic AMP were mediated by separate pathways. Alternatively, it could be argued that since the lactotrophs represent only about 20% of the anterior pituitary cells a small change in basal cyclic AMP concentration in the lactotrophs may not have been sufficient to significantly affect the measurements of total pituitary cyclic AMP content at the low dopamine concentrations.

According to the hypothesis outlined in the introduction, the dopamine inhibition of prolactin secretion would be mediated through a lowering of cyclic AMP concentration in the lactotrophs. To test this proposal we investigated the possibility that the actions of dopamine on secretion might be partially or totally antagonized by agents that could increase or maintain an elevated concentration of cyclic AMP in the lactotrophs. The results from these studies do not appear to be consistent with a role for cyclic AMP in the control of secretion by dopamine.

First, experiments with phosphodiesterase inhibitors showed that in the presence of dopamine, both theophylline and isobutylmethylxanthine could stimulate pituitary cyclic AMP accumulation well above the concentrations measured in control glands. Neither of these compounds, however, was able to block or even significantly decrease the inhibition by dopamine of prolactin secretion measured in the same incubations. In particular,

studies with theophylline indicated that the dopamine inhibition of prolactin secretion occurred without any significant attenuation of pituitary cyclic AMP concentrations. This result contrasts with a previous study by Kimura *et al.* (1976), who reported that dopamine was able to block a theophylline-induced increase in pituitary cyclic AMP concentration. The difference may be explained by the high concentration of dopamine ($100\mu\text{M}$) used to inhibit cyclic AMP accumulation in their experiments. The results obtained with isobutylmethylxanthine do not entirely exclude an involvement of cyclic AMP in the dopamine inhibition of prolactin secretion, since dopamine partially antagonized the isobutylmethylxanthine-induced increase in total pituitary cyclic AMP accumulation. It could be argued in this case that specific dopamine inhibition of cyclic AMP accumulation in the lactotrophs might be sufficient to explain the concomitant inhibition of secretion.

Secondly, exogenous addition of cyclic AMP derivatives, which should have blocked or decreased the effects of a dopamine-mediated inhibition of cellular cyclic AMP accumulation, produced no change in the inhibition of prolactin secretion whatsoever. The fact that the 8-bromo derivative of cyclic AMP by itself significantly stimulated both prolactin and somatotropin secretion provides evidence that the compound was able to penetrate these secretory cells. Although the evidence for dibutyryl cyclic AMP is less conclusive, these results are clearly in conflict with the proposed role for cyclic AMP in dopamine action. The results obtained with dibutyryl cyclic AMP contrast with several previous studies (Lemay & Labrie, 1972; Dannies *et al.*, 1976; Hill *et al.*, 1976). In particular, Hill *et al.* (1976) observed a stimulation of prolactin secretion by dibutyryl cyclic AMP and suggested that at concentrations of dopamine below $0.1\mu\text{M}$ the inhibition of prolactin secretion may be reversed by this derivative. On the basis of our results it seems possible that the inhibitory effects of higher dopamine concentrations may not be reversed by increasing cyclic AMP. The reason why dibutyryl cyclic AMP failed to stimulate secretion in the present study is unknown; however, inconsistent results have been noted previously using this compound (Hill *et al.*, 1976).

The third type of experiment tested the inhibitory effectiveness of dopamine under conditions where pituitary cyclic AMP accumulation was increased by cholera toxin. We have found that the substantial accumulation of cyclic AMP stimulated by this toxin was unchanged during incubations with dopamine and that this increase had no significant effect on the dopamine inhibition of prolactin secretion. In the present study cholera toxin alone had no effect on pituitary prolactin secretion;

previous investigators have reported, however, that this agent stimulated prolactin secretion from dispersed rat pituitary cells in culture (Rappaport & Grant, 1974). It seems possible therefore that cholera toxin has some effect on lactotrophs presumably through the activation of an adenylate cyclase system.

These results suggest that the dopamine inhibition of prolactin secretion may be independent of changes in cellular cyclic AMP concentrations in the lactotrophs. At the same time, the data obtained in the time-course studies and with the phosphodiesterase inhibitor isobutylmethylxanthine indicate that dopamine can affect pituitary cyclic AMP accumulation. Thus dopamine may have separate effects on prolactin secretion and cyclic AMP accumulation. It should be noted that the effects of these agents on pituitary cyclic AMP concentrations reflect the changes occurring in a number of different cell types and that a specific action of dopamine on cyclic AMP concentration in the prolactin-secreting cells has not been demonstrated. The possibility exists that dopamine may affect cyclic AMP concentration in pituitary cells other than the lactotrophs. Further investigations with purified lactotrophs are required to test this possibility. The mechanism whereby dopamine-receptor binding is linked to the inhibition of prolactin secretion is unknown, although previous studies suggest that the inhibition of cyclic AMP accumulation may be effected by a direct inhibition of adenylate cyclase activity (De Camilli *et al.*, 1979). The possibility that cyclic AMP may have some role in the regulation of prolactin secretion cannot be excluded, since 8-bromo cyclic AMP significantly stimulated the release of this hormone. Further, most of the studies carried out so far have used a concentration of dopamine that inhibits prolactin secretion maximally; modulation of the inhibitory actions of lower dopamine concentrations by agents such as isobutylmethylxanthine and theophylline has not yet been excluded in all cases.

The measurements of somatotropin secretion in the present study have provided useful controls to illustrate both the selective inhibitory action of dopamine for prolactin secretion and the effectiveness of various modulators of cyclic AMP metabolism to stimulate the secretion of a particular hormone. Dopamine had no significant effect on either basal or stimulated somatotropin secretion, under any of the conditions tested. The fact that all the agents tested that increased pituitary cyclic AMP accumulation also increased somatotropin secretion supports the idea that cyclic AMP is involved in the secretion of this hormone (Schofield, 1970).

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