ethanol and 10 ml toluene phosphor. Results were expressed as dpm/mg of tissue in the corresponding chromatographic zone; differences between duplicates were less than 15%. Except for the origin, each value was corrected for the radioactivity of the blank. Blank radioactivities expressed as dpm were: 5-hydroxyindoleacetic acid: 105 ± 20; 5-methoxyindoleacetic acid: 30 ± 10; 5-hydroxytryptophol: 25 ± 8; 5-methoxytryptophol: 29 ± 7; N-acetylserotonin: 25 ± 6; melatonin: 30 ± 10. Validation of this procedure by further TLC in various solvent system was published elsewhere[8]. In a 2nd experiment, 5 pineal glands per flask were incubated as above in the presence of none, 1 or 1000 nM estradiol and of 0.2 mg/ml puromycin. The conversion of ^{14}C serotonin to hydroxy- and methoxyindoles was determined as in experiment 1.

Results. As described previously[2] estradiol in the nmolar range enhanced serotonin conversion to 5-methoxy derivatives, particularly 5-methoxytryptophol and melatonin (table 1). In contrast concentrations of estradiol 1000 times greater decreased serotonin metabolism to both derivatives ($p < 0.05$). The stimulatory but not the inhibitory effect of estradiol on the in vitro metabolism of serotonin by pineal glands was blocked by adding puromycin to the culture medium (table 2). Puromycin per se did not affect serotonin metabolism significantly in this preparation.

Discussion. Foregoing data indicate that, as in vivo[2,6], the in vitro activity of estradiol on rat pineal gland exhibits a biphasic, dose-related pattern. In physiological concentrations estradiol enhanced the conversion of serotonin to the hormonally-active O-methylated derivatives 5-methoxytryptophol and melatonin, whereas greater, pharmacological, amounts impaired that metabolic reaction. The stimulatory effect of estradiol observed at physiological concentrations was blocked by puromycin, supporting the view that the translocation of estradiol-receptor complexes from the cytoplasm to the nuclei observed in incubated pineals[2] eventually results in genomic activation of pineal cells. In contrast to the stimulation that followed low concentrations of estradiol in culture medium, the inhibition observed at high, pharmacological doses, was not affected by adding puromycin. Hence inhibition of 5-methoxyindole synthesis by a large dose of estradiol seems not to depend upon changes in protein synthesis. Recent observations on the O-methylation of estradiol to its 3-methyl-ether by a partially purified bovine pineal HIOMT offer a basis for interpreting our results[9]. Since HIOMT K_m values for estradiol and N-acetylserotonin were close, and since in the presence of estradiol the K_m for S-adenosylmethionine was about one order of magnitude lower than in the presence of N-acetylserotonin, the possibility that high doses of estradiol may inhibit O-methylation of 5-hydroxyindoles in vivo and in vitro in a competitive way should be considered.

1 This study was supported by grant No. 6638 from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

2 D.P. Cardinali, in: The Pineal Gland, VO1.1. Ed. R.J. Reiter. CRC Press, Boca Raton, FA, in press (1981).
3 D.P. Cardinali, Neuroendocrinology *24*, 333 (1977).
4 F. Mizobe and M. Kurokawa, Eur. J. Biochem. *66*, 193 (1976).
5 F. Mizobe and M. Kurokawa, FEBS Lett. *87*, 45 (1978).
6 D.P. Cardinali, C.A. Nagle and J.M. Rosner, Hormone Res. *5*, 304 (1974).
7 D.C. Klein and A. Notides, Analyt. Biochem. *31*, 480 (1969).
8 D.P. Cardinali and J.M. Rosner, J. Neurochem. *18*, 1769 (1971).
9 J. Weisz, L.V. O'Brien and T. Lloyd, Endocrinology *102*, 330 (1978).

Effect of apomorphine hydrochloride administration on serum concentrations of prolactin and growth hormone in cattle

R.C. Gorewit

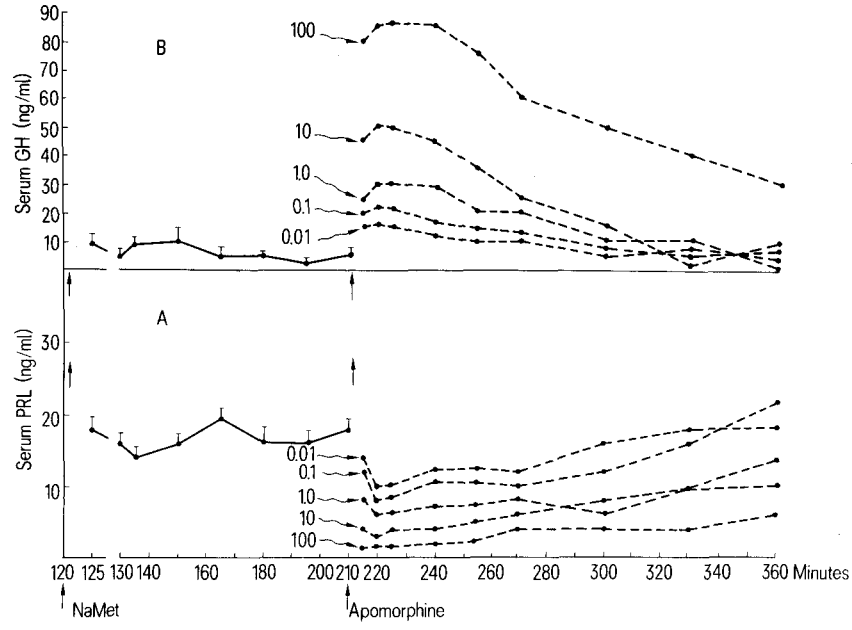
New York State College of Agriculture and Life Sciences, Cornell University, Ithaca (New York 14853, USA), 6 May 1980

Summary. Administration of apomorphine hydrochloride to cattle significantly depressed serum prolactin (PRL) concentrations and elevated serum growth hormone (GH) concentrations in a dosimetric fashion.

Prolactin (PRL) and growth hormone (GH) play important roles in regulating either the initiation or maintenance of lactation in cattle[1-3]. Growth hormone is also considered important in controlling growth processes of cattle[4]. Neuroendocrine mechanisms controlling pituitary hormone synthesis and release are obscure in ruminants, but have been extensively studied in laboratory animals. Dopamine (DA) and norepinephrine (NE) modulate secretion of GH[5] and PRL[6-10]. Studies with apomorphine hydrochloride, a selective DA receptor agonist, suggest that in humans, an inverse dopaminergic system exists controlling GH and PRL secretion[11,12]. The purpose of this investigation was to determine if such a system exists in cattle.

Materials and methods. 10 Holstein heifers (age 10–12 month) were used in this study. Body weights for animals ranged from 280 to 326 kg. Cattle were fitted with indwelling jugular cannulae and were allowed free choice to water during experimentation. Food was restricted 8 h prior to and during experimentation. On the day following cannulation, blood was collected and discarded for 2 h in order to accustom animals to experimental conditions. Blood samples were collected at 15 min intervals for 2 h prior to treatment (control). Immediately after the last sample was withdrawn, 1 ml of 0.1% sodium metabisulfite was administered over 1 min to all animals (control). Further blood samples were withdrawn at 5, 10, 15, 30, 45, 60, 75, and 90 min after sodium metabisulfite administration. After the last sample was withdrawn, cattle were divided into 5 groups, each consisting of 2 animals. Apomorphine hydrochloride (APM, dissolved in 0.1% sodium metabisulfite) was administered as described above. Cattle in group 1 received 0.01 µg/kg b.wt, group 2 received 0.1 µg/kg b.wt, group 3 received 1.0 µg/kg b.wt, group 4 received 10 µg/kg b.wt and group 5 received 100 µg/kg b.wt of APM. Blood was then collected at 5, 10, 15, 30, 45, 60, 90, 120 and 150 min after APM administration.

Bovine PRL was assayed by the method of Butler[13]. Serum GH was assayed using a double antibody radioimmunoassay, as described by Purchas et al.[14] with minor modifications. Bovine GH (NIH-GH-B18)[15] was used as the binding



Serum prolactin (A) and serum growth hormone (B) concentrations after 0.1% sodium metabisulfite (mean ± SEM, n = 10) and apomorphine hydrochloride (0.01, 0.1, 1.0, 10 and 100 µg/kg b.wt, n = 2 for each dosage).

antibody. Separation of antibody-bound GH from free GH was accomplished with sheep anti-rabbit-gamma globulin. Precipitates were counted in a gamma scintillation spectrometer after a 3 ml wash with ice cold phosphate buffered saline and centrifuged at 1000 × g.

Standard tubes contained from 0.1 to 5.0 ng NIH-GH-B18 and were assayed with each lot of unknowns. Serum aliquots of 200 µl were assayed in duplicate. Dilutions of bovine sera produced inhibition curves which closely paralleled those produced by standard (NIH-GH-B18) hormone preparation diluted in buffer (not shown). Recoveries of known amounts of standard bovine GH added to sera ranged from 96 to 101%. In 12 assays, the within assay coefficient of variation (SD/µl) averaged 2.8 ± 0.15 (mean ± SE) for 200, 250 and 300 µl of pooled serum. The between assay coefficient of variation was 6.2% ± 0.92 for 200, 250 and 300 µl vol. of serum pool, having a GH concentration of 4.0 ng/ml. Bovine thyroid stimulating hormone, luteinizing hormone, and PRL, at concentrations from 1 to 1000 ng, caused negligible reduction in binding of labelled GH.

Results. Bleeding had no effect on serum PRL concentrations, which averaged 14.3 ng/ml (not shown). Sodium metabisulfite administration did not affect serum concentrations of PRL, which averaged 16.5 ng/ml over 90 min (figure, a). However, APM administration reduced serum concentrations of PRL according to a dose response relationship. Concentrations of PRL were reduced within 5 min of APM administration, when compared with controls. Concentrations of PRL reached a nadir at 10 min after drug administration, when either 0.01, 0.1, 1.0 or 10 µg/kg APM was administered (figure, a). The nadir for PRL was achieved at 5 min post administration for the 100 µg/kg dose (figure, a). Apomorphine reduced serum concentrations of PRL for 120 min after it was given, at doses of 0.01 and 0.1 µg/kg. Resting concentrations of PRL were not achieved by 150 min post drug infusion, after the 1.0, 10.0 and 100 µg/kg drug doses were given (figure, a).

Bleeding had no affect on serum GH concentrations which averaged 6.5 ng/ml over 2 h (not shown). Administration of 0.1% sodium metabisulfite also had no affect on serum GH concentrations which averaged 6.6 ng/ml over 90 min (figure, b). Apomorphine administration elevated serum GH concentrations according to a dose response relationship. Serum GH was elevated within 5 min after each dose was administered (figure, b). Maximum concentrations of serum GH were achieved within 10 min after either 0.01, 0.1, 1.0 or 10 µg/kg of APM was administered. Peak concentrations of GH in sera, at this time, ranged from 15 to 50 ng/ml for the above doses. A dose of 100 µg/kg APM was most effective in elevating serum GH concentrations (figure, b). Administration of this dose elevated serum GH to 86 ng/ml, at peak within 15 min of administration. All doses of APM lead to prolonged elevation of serum GH concentrations. Growth hormone concentrations were maintained above resting levels, from 5 to 90 min, after doses of 0.01 to 1.0 µg/kg AMP were administered. Resting levels of serum GH were not achieved until 120 min post administration of 10 µg/kg AMP and 150 min post administration of 100 µg/kg APM.

Discussion. Serum concentrations of PRL in cattle were reduced after APM administration. The effect of APM on serum concentrations of PRL was also dose dependent. Apomorphine has been demonstrated to reduce circulating concentrations of PRL in rats[16] and man[17]. The drug was effective in reducing serum concentrations of PRL in cattle at a dose as low as 0.01 µg/kg.

Apomorphine hydrochloride elevated serum GH concentrations when it was given to cattle. The effect of APM on GH release was also dose dependent. Similar effects of APM on GH have been demonstrated in rats[16] and man[18]. The observation that serum GH was elevated after administration of 0.01 µg/kg apomorphine, indicates that small doses of DA agonists may stimulate GH release in ruminants. Hart and Cowie[19] have shown that i.v. administration of 10 mg of morphine significantly raised plasma GH levels in goats.

Although these data suggest an inverse dopaminergic control mechanism for GH and PRL release in cattle, the mode of apomorphine's action is unclear. Apomorphine may stimulate postsynaptic dopaminergic receptors which would enhance GH release. The drug might inhibit PRL release by stimulating these receptors, thereby increasing their inhibitory control over PRL release. Apomorphine could also affect GH and PRL release by direct action on the pituitary. Apomorphine has been shown to inhibit PRL release from isolated rat pituitaries[20].

Changes in GH and PRL release, produced as a result of APM administration, were not due to the emetic effect of the drug, because no emesis occurred in animals after drug treatment. Further work is needed to define the precise mechanism(s) whereby APM acts on GH and PRL release in cattle.

1 H.A. Tucker, in: Lactation, vol. 1, p. 277. Ed. B.L. Larson and V.R. Smith. Academic Press, New York 1974.
2 L.J. Machlin, Ed. F.C. Lu and J. Rendel. in: Anabolic agents in animal production, vol. 5, p. 43. Georg Thieme, Stuttgart 1975.
3 D. Lister, in: Patterns of growth and development in cattle, vol. 2, p. 341. Ed. H. Deboer and J. Martin. Martinus Nijhoff, Boston, 1978.
4 R.J. Heitzmann, in: Patterns of growth and development in cattle, vol. 2, p. 353. Ed. H. Deboer and J. Martin. Martinus Nijhoff, Boston 1978.
5 E.E. Muller, in: Frontiers in catecholamine research, p. 53. Ed. E. Usden and S. Snyder. Pergammon Press, Toronto 1973.
6 A.O. Donoso, W. Bishop, C.P. Fawcett, L. Krulich and M. McCann, Endocrinology *89*, 774 (1971).
7 I.A. Kamberi, R.S. Mical and J.C. Porter, Endocrinology *89*, 1042 (1971).
8 I.A. Kamberi, R.S. Mical and J.C. Porter, Endocrinology *88*, 1012 (1971).
9 C.J. Shaar and J.A. Clemens, Endocrinology *95*, 1202 (1974).
10 R.M. Macleod and J.E. Lehmeyer, Endocrinology *94*, 1077 (1974).
11 S. Lal, C.E. de la Vega, T.L. Sourkes and H.G. Friesen, J. clin. Endocr. Metab. *37*, 719 (1973).
12 J.B. Martin, S. Lal, G. Tolis and H.G. Friesen, J. clin. Endocr. Metab. *39*, 180 (1974).
13 W.R. Butler, P.V. Malven, L.B. Willett and D.J. Bolt, Endocrinology *91*, 793 (1972).
14 R.W. Purchas, K.L. MacMillan and H.D. Hafs, J. anim. Sci. *31*, 358 (1970).
15 Kindly supplied by National Institutes of Health (NIAMDD), Bethesda, MD, USA.
16 G.P. Mueller, J. Simpkins, J. Meites and K.E. Moore, Neuroendocrinology *20*, 121 (1976).
17 J.B. Martin, S. Lal, G. Tolis and H.G. Friesen, J. clin. Endocr. Metab. *39*, 180 (1974).
18 J.T. Rossa, R. Agrin and J.C. Melby, Am. J. Med. *63*, 909 (1977).
19 I.C. Hart and A.T. Cowie, J. Endocrinology *77*, 16 (1978).
20 E.G. Smalstig, B.D. Sawyer and J.A. Clemens, Endocrinology *95*, 123 (1974).

PRO EXPERIMENTIS

Simultaneous measurement of pyridine nucleotide fluorescence and field potentials from the olfactory cortical slice of the guinea-pig

S. Ji[1], T. Fujii and D.W. Lübbers

Max-Planck-Institut für Systemphysiologie, Rheinlanddamm 201, D-4600 Dortmund 1 (Federal Republic of Germany), *4 March 1980*

Summary. A new probe (tip diameter = 700 µm) consisting of a micro-light guide and a silver wire electrode was constructed to measure pyridine nucleotide fluorescence and field potentials from nerve tissues. Good kinetic correlations were found between the changes in field potentials and tissue pyridine nucleotide fluorescence caused by stoppage or by resumption of superfusion.

Since Chance and his co-workers introduced the tissue pyridine nucleotide (NADH) fluorometric technique in the late 1950's[2], several investigators have applied the method to the study of biochemical events occurring in nerve tissues under electrically-induced depolarizing conditions[3-7]. The present study was undertaken to measure simultaneously the electrical activity and the NADH fluorescence changes from the olfactory cortical slice of the guinea-pig caused by stoppage or resumption of superfusion. For this purpose we have developed a new probe consisting of a microlight guide[8,9] and a Ag-AgCl electrode (figure 1, A). Using this combination probe, we were able to measure the field potential and NADH fluorescence signals simultaneously and from the same tissue areas approximately 400 µm in diameter (figure 1, A).

Materials and methods. The method for producing the combination probe is basically the same as that for constructing a micro-light guide[8,9]. Figure 1, A shows schematically the structure of the combination probe. The Ag-AgCl wire was carefully insulated from the steel tubing. The optical fibres were connected to a 'DC' fluorometer[10] of the Johnson Research Foundation, University of Pennsylvania, and the silver wire to an oscilloscope.

The brain slices (0.4-0.5 mm thick) were prepared from the olfactory cortex of guinea-pigs, and the field potentials were measured under the 'gas-blow and medium-flow' conditions developed by Fujii et al.[11,12]. In order to stabilize the optical signal, it was necessary to place a strip of black polyethylene membrane (0.13 mm × 1.2 mm × 18 mm) beneath the brain slice as shown in figure 1, B, and to position the tip of the combination probe on that portion of the brain slice whose opposite surface was in contact with the black membrane. This arrangement eliminated the fluctuation of the NADH fluorescence signal which was apparently caused by periodic variations of the thickness of the Krebs-Ringer solution layer underneath the brain slice. Supramaximal stimulation was applied to the anterior part of the lateral olfactory tract (LOT). The temperature of the brain slice was continuously measured with a thermistor (0.7 mm in diameter, insulated with thin glass). The composition of the incubation medium (Krebs-Ringer's solution) was (mM): NaCl (120); KCl (4.8); KH_2PO_4 (1.2); $MgSO_4$ (1.2); $CaCl_2$ (2.6); $NaHCO_3$ (26); glucose (10); pH adjusted to 7.4 with bicarbonate buffer. The medium was equilibrated with a 5% CO_2 and 95% O_2 gas mixture.

Results. It was found that the NADH fluorescence intensity of the brain slice was very sensitive to temperature alterations. Figure 2 shows a typical temperature sensitivity curve of the olfactory cortical slice. From 4 brain slices, the average tissue NADH fluorescence intensity was found to be 101.9 ± 0.4% (mean ± SD) at 35 °C (relative to the fluorescence intensity at 37 °C set at 100%), 107 ± 1.3% at 30 °C, 114.2 ± 2.0% at 25 °C, and 121.2 ± 1.5% at 20 °C. The high temperature sensitivity of the NADH fluorescence intensity is not unique to the brain slice, since similar temperature sensitivity was also observed with isolated, hemoglobin-free perfused rat liver and an aqueous solution of NADH (S. Ji and T. Fujii, unpublished observations).