The effect of ovarian steroids on the uptake of ^{3}H-noradrenaline, ^{3}H-dopamine and ^{3}H-5-hydroxytryptamine by hypothalamic tissue *in vitro*

C. A. ENDERSBY and CATHERINE WILSON*

Pharmacology Department, Chelsea College of Science and Technology, London; Physiology Department, The Royal Veterinary College, London

Previous studies have shown that treatment of castrated rats with oestrogen and progesterone reduces the levels, turnover and synthesis rates of hypothalamic catecholamines, and inhibits release of pituitary gonadotrophins (Coppola, 1971; Bapna, Neff & Costa, 1971). Conversely catecholamine levels and turnover rates rise when gonadotrophin release is enhanced. (Coppola, 1969).

Uptake of amines by hypothalamic slices taken from rats under different endocrine conditions has now been investigated.

Ovariectomized adult Wistar rats were treated subcutaneously for three days with either oestradiol [(1 μg/rat)/day], progesterone [(4 mg/rat)/day] or progesterone [(4 mg/rat)/day] plus oestradiol [(0·05 μg/rat)/day]. The rats were killed and 3–4 hypothalami pooled. 7–9 experiments were carried out on each pool. Untreated ovariectomized rats served as controls.

5 mg of sliced hypothalami were pre-incubated at 27° C in 4 ml of oxygenated Krebs solution (containing 20 mg/ml ascorbic acid). The ^{3}H-noradrenaline (NA), ^{3}H-dopamine (DA) or ^{3}H-5-HT were added to give a final concentration of 5×10^{-8}M and incubation continued for 30 minutes. Radio-activity was measured in the filtered, washed slices.

The uptake of ^{3}H-5-HT was not altered by any of the steroid treatments. The uptake of ^{3}H-DA was reduced by 43·9% ($P<0·001$) in hypothalami taken from rats treated with progesterone plus oestradiol; treatment with progesterone or oestradiol alone had no effect. The uptake of ^{3}H-NA was increased by 83·7% ($P<0·005$) after treatment with oestradiol; progesterone or progesterone plus oestradiol had no effect.

Amine uptake was also studied at particular times before ovulation. Immature Wistar rats, kept in a lighting system of 14 h light and 10 h dark, were induced to ovulate with 20 I.U. Pregnant Mares Serum (PMS) on day 30. In these animals the gonadotrophin surge necessary for ovulation occurs at 18.00 hours on day 32, and the critical period of hypothalamic stimulation of the pituitary occurs between 14.00 hours and 16.00 hours (Endersby, Gallagher, Horth, McDonald & Wilson, 1972; Strauss & Meyer, 1962).

Groups of six PMS treated rats were killed at 2 hourly intervals from 10.00 hours to 22.00 hours on day 32, and the uptake of the three ^{3}H-amines noted on the pooled hypothalami. Control hypothalami were taken from untreated 32 day old rats.

The uptake of ^{3}H-5HT and ^{3}H-NA on day 32 did not differ between the PMS treated and control groups. However, both groups showed significant changes ($P<0·001$) throughout the day for both amines. The greatest uptake of ^{3}H-5-HT occurred at 16.00 hours and of ^{3}H-NA at 12.00 hours.

The uptake of ^{3}H-dopamine by hypothalami from PMS treated rats was not significantly different from the controls at 10.00, 12.00 and 18.00 hours, but was 21% greater ($P<0·05$) at 14.00 hours and 23·8% ($P<0·005$) less at 16.00 hours. It is possible that the duration of the critical period is controlled by changes in uptake of dopamine.

We are grateful to the Population Council, NY, and the Royal Society, London, for their support; and to Professor J. M. Robson for his advice.

REFERENCES

BAPNA, J., NEFF, N. H. & COSTA, E. (1971). A method for studying norepinephrine and serotonin metabolism in small regions of rat brain: Effect of ovariectomy on amine metabolism in anterior and posterior hypothalamus. *Endocrinology*, **89**, 1345–1349.

COPPOLA, J. A. (1969). Turnover of hypothalamic catecholamines during various states of gonadotrophin secretion. *Neuroendocrinology*, **5**, 75–80.

COPPOLA, J. A. (1971). Brain catecholamines and gonadotrophin secretion. Frontiers in Neuroendocrinology. pp. 129–143. ed. *Martini, L.* and *Ganong, W. F.*

ENDERSBY, C. A., GALLAGHER, M., HORTH, C. E., MCDONALD, P. G. & WILSON, C. (1972). Changes in plasma levels of oestrogen, progesterone and LH in immature rats treated with PMS. *J. Endocr.* (in press).

STRAUSS, W. F. & MEYER, R. K. (1962). Neural timing of ovulation in immature rats treated with gonadotrophin. *Science*, **137**, 860–861.

Is cortical dopamine only the precursor of noradrenaline?

G. BLANC, J. GLOWINSKI, L. STINUS and A. M. THIERRY* (introduced by L. L. IVERSEN)

Groupe NB, Laboratoire de Biologie Moleculaire, College de France, 11 Place Marcelin Berthelot, Paris 5e, France

It has generally been assumed that the catecholamine nerve terminals in the rat cerebral cortex were noradrenergic (Ungerstedt, 1971). However, the dopamine (DA) concentration in this structure is comparable to that of noradrenaline (NA). To investigate whether cortical DA might play a role other than merely the precursor of NA in noradrenergic neurons, NA and DA were estimated biochemically in the cortex (Thierry, *et al.*, 1971) after electrolytic or chemical destruction of the ascending noradrenergic pathways. Groups of 8 Charles River male rats were killed 5 weeks after either bilateral electrolytic lesions of the locus coeruleus or microinjections of 6-hydroxydopamine (6-OH-DA) made laterally to the pedunculus cerebellaris superior (PCS), or after sham operations. Electrolytic lesions were made with high frequency current (100 KH_3, 2 mA. 10 s). 6-OH-DA (2 μg in one μl, protected with ascorbic acid) was injected locally into the PCS (1 μl/5 min). Lesions of the dorsal noradrenergic pathway (locus coeruleus) or combined lesions of the ventral and dorsal noradrenergic pathways (PCS) induced marked decreases in cortical NA content (Table 1), of 65 and 92%

TABLE 1. *Catecholamine levels in the rat cortex after either bilateral electrolytic lesions of the locus coeruleus or bilateral microinjection of 6-OH-DA made laterally to the pedunculus cerebellaris superior (P.C.S.)*

	Locus coeruleus		P.C.S.	
	Control	Lesion	Control	6-OH-DA
NA (μg/g)	0·280 ± 0·014	0·097 ± 0·001*	0·213 ± 0·021	0·017 ± 0·003*
DA (μg/g)	0·196 ± 0·024	0·151 ± 0·009	0·122 ± 0·007	0·140 ± 0·029

Results are the mean ± S.E.M. of data obtained with 8 rats.
*$P < 0·001$ when compared with control values.

respectively. Surprisingly, cortical DA concentration was not changed significantly after either type of lesion to the noradrenergic neuronal systems. These results strongly suggest that most cortical DA is not localized in noradrenergic nerve terminals and this may suggest the existence of dopaminergic neurons in the cerebral cortex.

REFERENCES

THIERRY, A. M., BLANC, G. & GLOWINSKI, J. (1971). Dopamine-norepinephrine: another regulatory step of norepinephrine synthesis in central noradrenergic neurons. *Eur. J. Pharmacol.*, **14**, 303–307.

UNGERSTEDT, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta Physiol. Scand. Supplementum*, **367**, 1–48.

Pharmacological interactions between γ-hydroxybutyric acid and agents which modify cerebral γ-aminobutyric acid (GABA) metabolism

D. BENTON*, J. T. RICK and P. V. TABERNER (introduced by J. F. MITCHELL)

Department of Psychology, University of Birmingham, Birmingham 15 and Department of Pharmacology, University of Bristol Medical School, Bristol 8

γ-Hydroxybutyric acid (GHB) and imidazoleacetic acid (IMA) are both naturally occurring brain metabolites which, when administered to rats and mice at 400 mg/kg I.P.,