lysergic acid amide derivate, methysergide, suppressed prolactin secretion in the rat, probably only after its metabolic conversion[9], but central dopamine metabolism was not studied. It is difficult to assume that such a metabolic conversion (demethylation) is responsible for the differential effects observed with 32-085.

1 J.M. Vigouret, H.R. Bürki, A.L. Jaton, P.E. Züger and D.M. Loew, Pharmacology *16*, Suppl. 1, 156 (1978).
2 D.M. Loew, J.M. Vigouret and A.L. Jaton, in: Dopaminergic Ergot Derivatives and Motor Function, p. 129 Ed. K. Fuxe and D.B. Calne. Pergamon Press, Oxford 1979.
3 W. Kehr, R. Neumeister and R. Zimmermann, in: Dopaminergic Ergot Derivatives and Motor Function, p. 173. Ed. K. Fuxe and D.B. Calne. Pergamon Press, Oxford 1979.
4 E. Flückiger, U. Briner, W. Doepfner, E. Kovacs, P. Marbach and H.R. Wagner, Experientia *34*, 1330 (1978).
5 P. De Camilli, D. Macconi and A. Spada, Nature *278*, 252 (1979).
6 P.F. Spano and N.H. Neff, Analyt. Biochem. *42*, 113 (1971).
7 W. Wuttke and K.D. Döhler, in: Human Prolactin, p. 156. Ed. J.L. Pasteels and C. Robyn. Excerpta Medica, Amsterdam 1973.
8 E. Flückiger, in: Physiology of Mammary Glands, p. 71. Ed. A. Yokoyama, H. Mizuno and H. Nagasawa. Japan Scientific Press, Tokyo 1978.
9 S.W.J. Lamberts and R.M. MacLeod, Endocrinology *103*, 287 (1979).

Effect of orchidectomy and estradiol on acetylcholinesterase activity in rat brain areas and adenohypophysis

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Summary. Orchidectomy plus estradiol administration decreased acetylcholinesterase activity in the rat cerebral cortex and mesencephalon, while in the amygdala it was increased. In the adenohypophysis, orchidectomy increased enzyme activity, but subsequent estradiol treatment decreased it. The hypothalamus did not respond to either manipulation.

Cholinergic systems, which are known to play a significant role in normal autonomic neural function, can be modulated by many factors including hormones. Acetylcholine (ACh), the cholinergic neurotransmitter, has been implicated in the feedback regulation of gonadotropins[2]. Also, gonadal hormones play an important role in behavioural changes which are, in part, mediated through the cholinergic system of the CNS[3]. It is also known that acetylcholinesterase (AChE; EC 3.1.1.7), the hydrolytic enzyme for ACh, is of vital importance in the cholinergic neurohumoral transmission, while Moudgil and Kanungo[4] have shown that estradiol (E_2) increases AChE activity in the cerebral hemisphere and cerebellum of immature and adult female rats, at 4 h post-injection. In order to expand these observations, and also to check the influence of sex, we studied the effect of orchidectomy and E_2 on AChE activity in 4 brain areas, and the adenohypophysis in the male.

Material and methods. Adult male Wistar rats weighing 200–300 g were used. The animals were kept at 20–24 °C under a 12 h light period, starting from 07.00 h followed by a 12-h dark period, and fed standard pellet diet and water ad libitum. They were randomly divided into 3 groups of 8–10 animals; group 1: intact, group 2: orchidectomized (orchidex.) under ether anesthesia and used one or more weeks after, and group 3: orchidex. and given a single i.p. injection of 10 µg of E_2 (17 beta-estradiol; Sigma) per 100 g b.wt in 1 ml of 0.9% saline and used 4 h after the injection. The rats were killed by decapitation at a fixed time of the day (12.00 h) and the brains were quickly removed, freed of meninges and superficial blood vessels, and dissected on ice according to Gispen et al.[5] into: parietal cerebral cortex, mesencephalon, amygdala with overlying cortex pyriformis and hypothalamus. After removal of the brain from the skull case the neurohypophysis was separated in situ, and the adenohypophysis was removed from the sella turcica. A 2% homogenate (w/v) was prepared in 0.1 M ice-cold phosphate buffer (PB), pH 7.4, and AChE was assayed immediately by the spectrophotometric method of Ellman et al.[6] with minor modifications. In essence, the reaction mixture, in quartz cuvettes, contained 2.6 ml of PB, 0.4 ml of homogenate, 0.1 ml of dithiobisnitrobenzoate (0.01 M; Sigma), and 0.02 ml of acetylthiocholine iodide (0.075 M; Sigma) as substrate. The reaction was started by adding the substrate and the increase in absorbance was measured at 405 nm. The blank contained all the reactants except the substrate, and the AChE activity was calculated as µmole of substrate hydrolized/min/g of tissue. The results obtained were subjected to Student's t-test and are summarized in the table.

Results and discussion. It was found that orchidectomy plus E_2 significantly decreased AChE activity in the cerebral cortex and mesencephalon, while in the amygdala the activity was increased. In the adenohypophysis, orchidectomy drastically increased AChE activity, but subsequent E_2

AChE activity in male rat brain areas and adenohypophysis (average rates of hydrolysis of acetylthiocholine)

Area	Rates (µmole/min/g of tissue ± SEM)		
	Group 1 (n = 8) intact	Group 2 (n = 10) orchidex.	Group 3 (n = 8) orchidex. + E_2
Cerebral cortex	5.76 ± 0.15	5.73 ± 0.98	4.83 ± 0.39*
Mesencephalon	13.01 ± 0.71	10.83 ± 1.52	9.78 ± 1.50*
Amygdala	8.06 ± 1.24	6.88 ± 0.78***	10.44 ± 0.78*
Hypothalamus	7.68 ± 0.15	6.92 ± 1.54	6.58 ± 0.95
Adenohypophysis	1.03 ± 0.25**	3.93 ± 0.96***	1.35 ± 0.19

* Difference from group 1: significant ($p < 0.05$); ** Difference from group 2: significant ($p < 0.05$); *** Difference from group 3: significant ($p < 0.05$).

treatment decreased it. Unlike the other 3 brain areas studied, the hypothalamus appears to be indifferent to orchidectomy, with or without E_2 administration.

Our observations on the effect of orchidectomy followed by E_2 injection on the AChE activity in the cerebral cortex are contrary to the findings of James and Kanungo[7] who claimed that a similar dose of E_2 causes an increase in AChE activity at all ages. This discrepancy may be due to the fact that they used the whole cerebral hemisphere as against the cortex in our study. The response of the amygdala, on the other hand, seems to agree with Kamberi's preliminary report that AChE activity is diminished by gonadectomy in the adult rat and augmented by E_2 replacement therapy[8]. Although gonadectomy decreases the enzyme activity in young[7] and adult rats[7,8], we did not observe any decrease in all the brain areas studied. This may be due to the slight differences in the ages at gonadectomy. We, however, noticed a significant increase in the AChE activity in the adenohypophysis.

From previous demonstrations by autoradiography[9], and binding studies[10] that sex steroids act in the hypophysis, preoptic area, hypothalamus and amygdala we may speculate, from our results, that orchidectomy and E_2 administration could regulate AChE activity by facilitatory and inhibitory mechanisms at different target sites. It is suggested, then, that the hormonal regulation of AChE may play a significant role in neuroendocrinological and behavioural mammalian events.

1 Department of Biochemistry, University of Ibadan, Ibadan (Nigeria).
2 J.W. Everett, Physiol. Rev. *44*, 373 (1964).
3 A. Oliverio, B.E. Eleftheriou and D.W. Bailey, Physiol. Behav. *10*, 893 (1973).
4 V.K. Moudgil and M.S. Kanungo, Biochem. biophys. Res. Commun. *52*, 725 (1973).
5 W.H. Gispen, P. Schotman and E.R. De Kloet, Neuroendocrinology *9*, 285 (1972).
6 G.L. Ellman, K.D. Courtney, V. Andres, Jr and R.M. Featherstone, Biochem. Pharmac. *7*, 88 (1961).
7 T.C. James and M.S. Kanungo, Biochim. biophys. Acta *538*, 205 (1978).
8 I.A. Kamberi, in: Schering Workshop on Central Actions of Estrogenic Hormones, p.48. Ed. G. Raspe. Pergamon Press, Oxford 1975.
9 W.E. Stumpf and M. Sar, in: Receptors and Mechanism of Action of Steroid Hormones, part 1, p.41. Ed. J.R. Pasqualini. M. Dekker, New York 1976.
10 B.S. McEwen, J.L. Gerlach, V.N. Luine and I. Lieberburg, Psychoneuroendocrinology *2*, 249 (1977).

The induction of diabetes in rats by intramuscular administration of streptozotocin[1]

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Summary. Streptozotocin administered i.m. was as effective a diabetogenic agent as when administered i.v. This is useful, particularly for the induction of diabetes in small animals.

As part of a study on the hypoglycaemic properties of some local remedies, experimental diabetes was induced in rats with streptozotocin. Following difficulties encountered with i.v. administration to small animals the drug was injected into the thigh muscles of the leg and its effects studied. Streptozotocin given i.m. produced diabetes of dose dependent severity similar to its effects after i.v. administration as reported by Junod et al.[3].

Materials and methods. Male albino Sprague Dawley rats weighing between 100 and 300 g were used. The experimental groups were matched for weight for each set of experiments.

For the induction of diabetes the animals were fasted for 16 h. Individual doses of pure streptozotocin (Upjohn Research Labs, USA) were prepared by weighing and immediately before injection dissolved in 1 ml citrate buffer pH 4.5. Following the injection, the animals were allowed food and water ad libitum for the remainder of the experiment.

Blood samples for glucose determination were taken from a

Table 1. Severity of hyperglycaemia in rats 24 h after streptozotocin administration

Dose mg/kg wt	No. of rats studied	Degree of hyperglycaemia induced** Severe	Moderate	Mild	None	No. with glycosuria	No. with ketonuria	Average survival rate after 10 days
120 mg	7	7	-	-	-	7 (100%)	7 (100%)	0%
60 mg	13	13	-	-	-	13 (100%)	13 (100%)	46%
45 mg	60	60	-	-	-	60 (100%)	12 (20%)	100%
40 mg	60	46	6	4	4	60 (100%)	12 (20%)	100%
30 mg	50	10	25	3	12	35 (70%)	2 (4%)	100%
20 mg	21	-	-	-	21	12* (57%)	0	100%

*Transient glycosuria; **Mild = > 140 mg and < 200 mg%; Moderate = > 200 mg and < 300 mg%; severe = > 300 mg%.

Table 2. Plasma insulin of rats injected with 30 mg/kg streptozotocin

	Random blood glucose (mg%)	Insulin (μU/ml)	No. of animals
Normoglycaemic from start of experiment	100 ± 5.3*	49 ± 5.9	12
Normoglycaemic after 3 to 21 days	101 ± 8.2	28 ± 4.6	9

*Mean ± SEM.