Brit. J. Pharmacol. (1966), **26**, 601–605.

A STUDY OF CENTRAL ADRENERGIC MECHANISMS IN THE REGULATION OF THE OESTROUS CYCLE OF ALBINO MICE

BY

K. P. BHARGAVA AND M. L. GUPTA

From the Department of Pharmacology and Therapeutics, K.G. Medical College, Lucknow University, Lucknow 3, India

(*Received October* 29, 1965)

Several centrally acting drugs have been reported to prolong the oestrous cycle in laboratory animals. These include reserpine, chlorpromazine and several other phenothiazine tranquillizers (Bhargava & Jaitly, 1964), and ergot alkaloids, for example ergotoxine (Shelesnyak, 1955). These drugs either deplete catecholamine stores (Carlsson, Rosengren, Bertler & Nilsson, 1957) or may have a blocking effect on the catecholamine receptors in the hypothalamus (Das Gupta, Mukerjee & Werner, 1954; Dell, 1960). Robson (1932) has reported that adrenaline inhibits the oestrous cycle in mice. Adrenaline has also been reported to induce ovulation when given intrahypophysially or intraventricularly (Markee, Everett & Sawyer, 1952). These findings suggest that catecholamines are involved in the release of the luteinizing hormone releasing factor. If this is true, then any change in the catecholamine level of the brain would affect the oestrous cycle. In the present study the effect of those drugs which either change the catecholamine level or block the catecholamine receptors in the brain has been studied on the oestrous cycle of albino mice.

METHODS

Female mice weighing from 18 to 32 g were used. They were given a diet containing wheat, yeast, cod liver oil (in the form of a paste) and green vegetables. The mice were screened for regularity of oestrous cycle for 20 days. Only those mice which showed regular oestrous cycle were selected for the study. Vaginal smears were made by the method used by Bhargava & Jaitly (1964). The smears were stained with Leishman's stain and examined under the microscope. The criteria used for differentiating the stages of the oestrous cycle were those of Allen (1922). Separate smear records were kept for each mouse.

All the drugs were dissolved in distilled water and injected intraperitoneally. The drugs were given daily. The concentrations of the drugs were such that a volume of 0.015 ml./g of body weight was injected into each mouse. Each drug was injected for 15 days and the vaginal smears during this period were compared with the smear records of the same mice for the 15 days immediately preceding the treatment with the drug. Each mouse, therefore, served as its own control.

The monoamine oxidase inhibitors were injected intraperitoneally, 1 hr before the administration of α-methylmetatyrosine into the mice receiving combined treatment.

RESULTS

The results are summarized in Table 1. Of the compounds tested, only α-methylmetatyrosine and imipramine prolonged the oestrous cycle and the results with these two drugs are highly significant. Tranylcypromine, pheniprazine and α-methyldopa

TABLE 1

THE EFFECT OF SOME AGENTS INFLUENCING CENTRAL ADRENERGIC MECHANISMS ON THE OESTROUS CYCLE OF UNTREATED AND MONOAMINE OXIDASE INHIBITOR-TREATED ALBINO MICE

Values are means with standard errors. *t*-Values from Student's *t*-test. *Pheniprazine prevented the prolongation of the oestrous cycle induced by α-methylmetatyrosine. The prolongation of oestrous cycle in the group treated with α-methylmetatyrosine is significantly different from the prolongation observed in the group treated with pheniprazine and α-methylmetatyrosine ($P < 0.05$)

Drug	No. of mice	Dose (mg/kg)	No. of cycles studied	Average duration (days) of oestrous cycle		*t*	*P*
				Before treatment	During treatment		
α-methylmetatyrosine	6	30	23	4·68±0·36	10·71±1·42	5·63	<0·001
Tranylcypromine+ α-methylmetatyrosine	7	2+ 30	26	5·73±0·46	6·00±0·38	0·409	<0·6
Pheniprazine*+ α-methylmetatyrosine	5	2+ 30	19	4·69±0·29	7·00±0·81	3·10	<0·01
Imipramine	7	15	28	4·82±0·68	10·33±1·55	3·72	<0·01
α-Methyldopa	7	150	22	5·16±0·43	6·7 ±1·129	1·29	<0·2
Tranylcypromine	5	2	20	4·77±0·75	7·28±1·08	1·84	<0·1
Pheniprazine	5	2	24	3·07±0·32	5·00±0·52	0·107	>0·9

had no significant effect on the duration of the oestrous cycle. Tranylcypromine and pheniprazine inhibited the prolongation of oestrous cycle induced by α-methylmetatyrosine in the mice.

DISCUSSION

A regular oestrous cycle is maintained by the harmonious functioning of a number of endocrine glands. The hypothalamus controls the production and/or release of gonadotrophic hormones from the pituitary gland (Aschner, 1912). Several workers have recently reported the presence of gonadotrophin releasing factors in hypothalamic extracts (Nikitovitch-Winer, 1962; Campbell, Feuer & Harris, 1964). These extracts contain luteinizing hormone releasing factor and follicle stimulating hormone releasing factor (Dhariwal, Nallar, Batt & McCann, 1965). There is a possibility that the neurohumoral mediator for the release of the luteinizing hormone releasing factor in the hypothalamus may be a catecholamine. We have provided results to support this hypothesis. α-Methylmetatyrosine in a dose of 10 mg/kg almost completely releases noradrenaline and some dopamine from the brain of mice without affecting the level of 5-hydroxytryptamine. A higher dose (40 mg/kg) predominantly depletes catecholamines with only a slight effect on 5-hydroxytryptamine (Costa, Gessa, Kuntzman & Brodie, 1962). We found that a dose of 30 mg/kg of α-methylmetatyrosine produces a highly significant prolongation of the oestrous cycle in albino mice. This effect may be due to depletion of catecholamines from the hypothalamus thus diminishing the stimulatory effect of the hypothalamus on the anterior pituitary for want of catecholamines. To test this hypothesis, α-methylmetatyrosine (30 mg/kg) was administered to mice which

had been previously treated with monoamine oxidase inhibitors, tranylcypromine (Green & Erickson, 1960) and pheniprazine (Brodie, Spector & Shore, 1959) (Table 1). These two drugs completely prevented the prolongation of oestrous cycle by α-methylmetatyrosine. This can be explained by the fact that α-methylmetatyrosine does not deplete brain noradrenaline in monoamine oxidase-inhibited mice (Costa, Gessa, Hirsch, Kuntzman & Brodie, 1962). Bovet-Nitti & Bignami (1962) have reported that the pseudopregnancy provoked by reserpine could be completely prevented by prior administration of some monoamine oxidase inhibitors but their study does not exclude 5-hydroxytryptamine as a possible neurohumour in the regulation of the oestrous cycle whereas our study excludes this possibility, as α-methylmetatyrosine is a specific depletor of catecholamines in the dose tested (Costa *et al.*, 1962b).

Imipramine in a dose of 15 mg/kg has also been found to prolong the oestrous cycle of albino mice in our study. Many studies, particularly those using relatively high doses of imipramine, indicate that it has a weak phenothiazine-like activity (Sigg, 1962). Several phenothiazine tranquillizers have been reported to prolong the oestrous cycle of albino mice (Bhargava & Jaitly, 1964). They also block the release of gonadotrophin in experimental animals and delay ovulation and menstruation in women (Campbell, 1963). The mechanism of action of imipramine might be the same as that of phenothiazine tranquillizers. Some of the central effects of phenothiazines have been attributed to their antiadrenaline activity (Bradley & Hance, 1957 ; Das Gupta & Werner, 1954). Imipramine also, in high doses (15 mg/kg), has been reported to have an adrenergic blocking activity on the peripheral autonomic system (Osborne & Sigg, 1960). The prolongation of the oestrous cycle in albino mice by imipramine may also be due to central adrenergic blockade.

Though α-methyldopa depletes tissues of noradrenaline (Porter, Totaro & Leiby, 1961) it failed to produce a significant effect on the oestrous cycle of albino mice even when given daily for 15 days. This can be explained on the basis of the observation that the responses to sympathetic stimulation are not markedly diminished by α-methyldopa (Goldberg, Dacosta & Ozaki, 1960 ; Stone, Ross, Wenger, Ludden, Blessing, Totaro & Porter, 1962 ; Day & Rand, 1964). Day & Rand (1963) suggested that α-methyldopa is metabolized in the body to yield α-methylnoradrenaline. The α-methylnoradrenaline formed may enter storage sites for noradrenaline at sympathetic nerve endings and then be released instead of noradrenaline as the sympathetic neurotransmitter. Similarly Carlsson & Lindqvist (1962) have suggested that α-methylated amines formed from α-methyldopa could take over the role of normal catecholamines in the central nervous system. Our results with α-methyldopa also support this hypothesis as α-methyldopa has no significant effect on the oestrous cycle.

The two monoamine oxidase inhibitors tranylcypromine and pheniprazine had no significant effect on the oestrous cycle of albino mice when given alone but they inhibited the α-methylmetatyrosine-induced prolongation of oestrous cycle in the albino mice when given before α-methylmetatyrosine (see Table 1). This can be explained by the fact that monoamine inhibitors increase the level of catecholamines in the brain (Green & Erickson, 1960 ; Brodie *et al.*, 1959) and thus will have no effect on the oestrous cycle. Poulson & Robson (1964) also could find no evidence that monoamine oxidase inhibitory activity of some of the compounds was related to their antifertility activity.

Our findings with monoamine oxidase inhibitors also support the hypothesis that an adrenergic component is involved in the regulation of the oestrous cycle in mice.

SUMMARY

1. The effect of some agents influencing central adrenergic mechanisms has been studied on the duration of oestrous cycle in albino mice.

2. α-Methylmetatyrosine and imipramine significantly prolonged the oestrous cycle.

3. The dopa decarboxylase inhibitor, α-methyldopa, and monoamine oxidase inhibitors had no effect on the oestrous cycle.

4. The effect of α-methylmetatyrosine on the oestrous cycle could be completely inhibited by prior treatment of mice with the monoamine oxidase inhibitors tranylcypromine and pheniprazine.

5. The results indicate that an adrenergic mechanism is involved in the release of neurohumoral transmitters regulating the oestrous cycle in albino mice.

This study was financed by the Indian Council of Medical Research, New Delhi. We gratefully acknowledge receiving α-methylmetatyrosine and α-methyldopa from Merck Sharp & Dohme, imipramine from Geigy, pheniprazine from Lake Side Laboratories and tranylcypromine from Smith Kline & French. We are thankful to Dr G. P. Gupta for his help in preparing this manuscript.

REFERENCES

ALLEN, E. (1922). The oestrous cycle in the mouse. *Amer. J. Anat.*, **30**, 297–348.

ASCHNER, B. (1912), *Wien. Klin. Wschr.*, **25**, 1042. Quoted by SAWYER, C. H. (1962). Mechanism by which drugs and hormones activate and block release of pituitary gonadotropins. *Proc. 1st Int. Pharmacol. Meeting*, ed. GUILLEMIN, R., vol. 1 pp. 27–46, London : Pergamon Press.

BHARGAVA, K. P. & JAITLY, K. D. (1964). The effect of some phenothiazine tranquillizers on the oestrous cycle of ablino mice. *Brit. J. Pharmacol.*, **22**, 162–165.

BOVET-NITTI, F. & BIGNAMI, G. (1962). Action de quelques dérivés de l'aminomethyl-2-Benzodioxanne-1, 4 sur l'equilibre hormonal du cycle de la pseudogrossesse et de la grossesse chez la ratte. *Proc. 1st Int. Pharmacol. Meeting*, vol. 1, pp. 131–149. London : Pergamon Press.

BRADLEY, P. B. & HANCE, A. J. (1957). The effect of chlorpromazine and methopromazine on the electrical activity of the brain in the cat. *Electroenceph clin. Neurophysiol.*, **9**, 191–215.

BRODIE, B. B., SPECTOR, S. & SHORE, P. A. (1959). Interaction of drugs with norepinephrine in the brain. *Pharmacol. Rev.*, **11**, 548–564.

CAMPBELL, H. J. (1963). Endocrine secretion and the central nervous system. In *Recent Advances in Physiology*, ed. CREESE, R., 8th ed., pp. 178–221. London : Churchill.

CAMPBELL, H. J., FEUER, G. & HARRIS, G. W. (1964). The effect of intrapituitary infusion of median eminence and other brain extracts on anterior pituitary gonadotrophic secretion. *J. Physiol. (Lond.)*, **170**, 474–486.

CARLSSON, A. & LINDQVIST, M. (1962). In vivo decarboxylation of α-methyl dopa and α-methyl metatyrosine. *Acta physiol. scand.*, **54**, 87-94.

CARLSSON, A., ROSENGREN, E., BERTLER, A. & NILSSON, J. (1957). Effect of reserpine on the metabolism of catecholamines. In *Pyschotropic Drugs*, ed. GARATTINI, S. & GHETTI, V., pp. 363–372. Amsterdam : Elsevier.

COSTA, E., GESSA, G. L., HIRSCH, C., KUNTZMAN, R. & BRODIE, B. B. (1962a). On current status of serotonin as a brain neurohormone and in action of reserpine-like drugs. *Ann. N.Y. Acad. Sci.*, **96**, 118-133.

COSTA, E., GESSA, G. L., KUNTZMAN, R. & BRODIE, B. B. (1962b). Effect of drugs on storage and release of serotonin and catecholamine in brain. *Proc. 1st Int. Pharmacol. Meeting*, ed. PATON, W. D. M., vol. 8, pp. 43–71. London : Pergamon Press.

DAS GUPTA, S. R., MUKERJEE, K. L. & WERNER, G. (1954). The activity of some central depressant drugs in acute decorticate and diencephalic preparations. *Arch. Int. Pharmacodyn.*, **97**, 149–156.

DAS GUPTA, S. R. & WERNER, G. (1954). Inhibition of hypothalmic medullary and reflex vasomotor responses by chlorpromazine. *Brit. J. Pharmacol.*, **9**, 389–391.

DAY, M. D. & RAND, M. J. (1963). A hypothesis for the mode of action of α-methyl dopa in relieving hypertension. *J. Pharm. Pharmacol.*, **15**, 221–224.

DAY, M. D. & RAND, M. J. (1964). Some observations on the pharmacology of α-methyldopa. *Brit. J. Pharmacol.*, **22**, 72–86.

DELL, P. (1960). Interaction of an adrenergic mechanism during brain stem reticular activation. In *Adrenergic Mechanisms*, ed. VANE, J. R., WOLSTENHOLME, G. E. W. & O'CONNOR, M., pp. 393–409. London : Churchill.

DHARIWAL, A. P. S., NALLAR, R., BATT, M. & MCCANN. S. M. (1965). Separation of follicle stimulatory hormone releasing factor from luteinizing hormone releasing factor. *Endocrinology*, **76**, 290–294.

GOLDBERG, L. I., DACOSTA, F. M. & OZAKI, M. (1960). Actions of decarboxylase inhibitor α-methyl-3, 4-dihydroxyphenylalanine in dog. *Nature (Lond.)*, **188**, 502–504.

GREEN, H. & ERICKSON, R. W. (1960). Effect of trans-2-phenyl cyclopropylamine upon norepinephrine concentration and monoamine oxidase activity of rat brain. *J. Pharmacol. exp. Ther.*, **129**, 237–242.

MARKEE, J. E., EVERETT, J. W. & SAWYER, C. H. (1952). The relationship of the nervous system to the release of gonadotrophin and the regulation of the sex cycle. In *Recent Progress in Hormone Research*, ed. PINCUS, G., vol. 7, pp. 139–163. New York : Academic Press.

NIKITOVITCH-WINER, M. B. (1962). Induction of ovulation in rats by direct intrapituitary infusion of median eminence extracts. *Endocrinology*, **70**, 350–358.

OSBORNE, M. & SIGG, E. B. (1960). Effect of imipramine on the peripheral autonomic system. *Arch. Int. Pharmacodyn.*, **129**, 273–289.

PORTER, C. C., TOTARO, J. A. & LEIBY, C. M. (1961). Some biochemical effects of α-methyl-3,4-dihydroxyphenyl-alanine and related compounds in mice. *J. Pharmacol. exp. Ther.*, **134**, 139–145.

POULSON, E. & ROBSON, J. M. (1964). Effect of phenelzine and some related compounds on pregnancy and on sexual development. *J. Endocr.* **30**, 205–215.

ROBSON, J. M. (1932). Adrenaline and oestrous cycle in the mouse. *Proc. Roy. Soc. Edinburgh*, **52**, 434–444.

SHELESNYAK, M. C. (1955). Disturbance of hormone balance in the female rat by a single injection of ergotoxine-ethane sulphonate. *Amer. J. Physiol.*, **180**, 47–49.

SIGG, E. B. (1962). The Pharmacodynamics of imipramine. In *Psychosomatic Medicine*, ed. NODINE, J. H. & MOYER, J. H., pp. 671–678. Philadelphia : Lea & Febriger.

STONE, C. A., ROSS, C. A., WENGER, H. C., LUDDEN, C. T., BLESSING, J. A., TOTARO, J. A. & PORTER, C. C. (1962). Effect of α-methyl-3, 4-dihydroxyphenylalanine (methyl dopa), reserpine and related agents on some vascular responses in the dog. *J. Pharmacol. Exp. Ther.*, **136**, 80–88.