Br. J. Pharmac. (1971), **41**, 187–197.

Effects of progesterone on cardiovascular responses to amines and to sympathetic stimulation in the pithed rat

J. R. FOZARD*

Department of Pharmacology, The University, Manchester M13 9PL

Summary

1. Blood pressure and heart rate responses to adrenaline, noradrenaline, tyramine, 5-hydroxytryptamine and stimulation of the spinal sympathetic outflow were measured in pithed rats pretreated either with progesterone (20 mg/kg daily for 14 days) or the vehicle solution of ethyl oleate.

2. Pretreatment with progesterone increased the durations but not the magnitudes of the blood pressure and heart rate responses to adrenaline and that phase of the response to sympathetic stimulation attributable to amine release from the adrenal medulla.

3. Responses to noradrenaline, tyramine, 5-hydroxytryptamine and that phase of the response to sympathetic stimulation associated with amine release from the sympathetic nerves were not significantly different in the two groups.

4. Pyrogallol (5 mg/kg) increased the duration but not the magnitude of responses to adrenaline, noradrenaline and sympathetic stimulation in both experimental groups. The increases in duration were consistently less in animals pretreated with progesterone than in controls.

5. Pretreatment with progesterone did not affect the total amount of radioactivity nor the proportion of catechol to non-catechol metabolites excreted in the urine during a period of 7·25 h following an intraperitoneal injection of (±) isoprenaline-7-^{3}H.

6. It is concluded that the effects of progesterone may result from a localized decrease in catechol O-methyl transferase activity within the cardiovascular system.

Introduction

Progesterone is an effective prophylactic agent in the treatment of migraine (Singh, Singh & Singh, 1947 ; Greene & Dalton, 1953 ; Greene, 1963), yet its mechanism of action remains obscure (Green, 1967). As migraine is a vascular phenomenon (Wolff, 1963), the results from two recent reports describing the effects of progesterone on vascular reactivity *in vitro* are of potential interest.

On the perfused rabbit auricular artery, progesterone enhanced responses to tryptamine and 5-hydroxytryptamine, depressed those to sympathetic nerve stimu-

* Present address: Pharmakologisches Institut der Universitat Mainz, 6500 MAINZ, Langenbeckstrasse 7, Germany.

lation and had no effect on responses to noradrenaline and tyramine (Fozard & Schnieden, 1970). In contrast, on the rabbit aortic strip, progesterone enhanced vasoconstrictor responses to a variety of sympathomimetic amines, including noradrenaline and tyramine (Kalsner, 1969a).

These conflicting observations *in vitro* prompted the present investigation in which the effects of progesterone on a variety of cardiovascular responses have been investigated *in vivo* using the pithed rat preparation.

Methods

Cardiovascular experiments

Female Wistar rats of 210–260 g body weight were used. Before pretreatment, the animals were randomly allocated to either a control or a test group. Animals in the test group (mean weight 232 g) were injected intramuscularly with progesterone (20 mg/kg) daily for 14 days. Those in the control group (mean weight 233 g) received similar daily injections of the vehicle solution (ethyl oleate).

Eighteen hours after the last dose, each rat was anaesthetized with pentobarbitone, pithed and set up for femoral intravenous injection of drugs as previously described (Fozard & Leach, 1968). Blood pressure was recorded from a common carotid artery using a Statham P23AC transducer. Heart rate was monitored using a Devices instantaneous ratemeter (Type 2750) triggered from the pulse pressure.

The sympathetic outflow of the spinal cord was stimulated by the method of Gillespie & Muir (1967) for 15 s with rectangular pulses of 80 V and 1 ms duration. Atropine (0·5 mg) and tubocurarine (0·5 mg) were given intraperitoneally immediately after the initial resting blood pressure and heart rate had been recorded.

Conduct of the experiments

Blood pressure and heart rate responses to adrenaline (0·025, 0·05, 0·1 μg), noradrenaline (0·025, 0·05, 0·1 μg), tyramine (2·5, 5, 10 μg), 5-hydroxytryptamine (1, 2, 4 μg) and stimulation of the spinal sympathetic outflow (1, 3, 6 Hz) were elicited in both control and progesterone pretreated pithed rats.

The results are the pooled observations from two separate experimental series. In the first, responses to noradrenaline, tyramine, 5-hydroxytryptamine and sympathetic stimulation were examined. On the basis of the results obtained, a second series was carried out in which dose-response curves to adrenaline, noradrenaline and sympathetic stimulation, were established. In this series, the effects of an inhibitor of catechol-O-methyl transferase were observed in both the control and progesterone groups by re-establishing responses to adrenaline (0·05 μg), noradrenaline (0·05 μg) and sympathetic stimulation (3 Hz) approximately 30 min after an intravenous injection of pyrogallol (5 mg/kg).

Expression of results

In all experiments the blood pressure responses to sympathetic stimulation were biphasic with an initial brief rise followed by a secondary more prolonged rise. The results were analysed in terms of both phases of the response.

The magnitude and duration of each pressor and heart rate response were recorded. For adrenaline, noradrenaline, tyramine, 5-hydroxytryptamine and the second

phase of the response to sympathetic stimulation, duration was expressed as the time for complete return to the preinjection level of blood pressure or heart rate. Because the first phase of the response to sympathetic stimulation did not return to preinjection levels, its duration was taken as the period between the end of stimulation and the onset of the second phase.

Radioactivity studies

Rats were treated with progesterone or ethyl oleate as described above. Eighteen hours after the last dose each rat was given 5 ml of distilled water by stomach tube and an intraperitoneal injection of 12·5 μCi (1·09 μg) of (±) isoprenaline-7-^{3}H. Urine was collected into ice-cooled tubes containing 0·5 ml of 4% di-sodium ethylenediaminetetracetic acid solution and 0·1 ml of 10% ascorbic acid solution. Urine was collected over 4·25 h and again, after administration of a further 5 ml of distilled water, over the next 3 hours. Each urine sample was diluted to 10 ml with distilled water and adjusted to pH 8·4 with 50% sodium acetate and 0·5 N sodium bicarbonate solutions.

Total urinary radioactivity was estimated by counting a 1 ml aliquot of the diluted urine. The catechol and non-catechol metabolites present in the urine were estimated by alumina column separation according to the methods of Weil-Malherbe & Bone (1952) and Kopin, Axelrod & Gordon (1961).

The procedure for the radioassay of tritium has been described in full by Foster (1968, 1969).

Statistical methods

All measures of variation of means quoted are standard errors. Student's *t* test was used to assess the significance of a difference between means.

Drugs

The following drugs were used; all doses are expressed as the free base. Atropine sulphate (BDH), (−)-adrenaline hydrogen tartrate (BDH), (−)-noradrenaline hydrogen tartrate (Koch-Light), 5-hydroxytryptamine creatinine sulphate (Koch-Light), tyramine hydrochloride (Koch-Light), progesterone (BDH), tubocurarine chloride (Burroughs Wellcome).

(±)-Isoprenaline-7-^{3}H (5·03 Ci/mmol in 0·1 N acetic acid) was supplied by the New England Nuclear Corporation and diluted with distilled water to yield a stock solution of 25 μCi/ml in 0·025 N acetic acid.

Results

Effects of progesterone on the mean resting systolic blood pressure and heart rate of the pithed rat

The mean resting blood pressure and heart rate of seventeen control pithed rats were 52·8 ± 1·8 mmHg (1 mmHg ≡ 1·333 mbar) and 202 ± 8 beats/min respectively. In sixteen pithed rats pretreated with progesterone (20 mg/kg) daily for 14 days, the equivalent values were 50·9 ± 2·1 mmHg and 204 ± 7 beats/min, respectively. The differences were not significant.

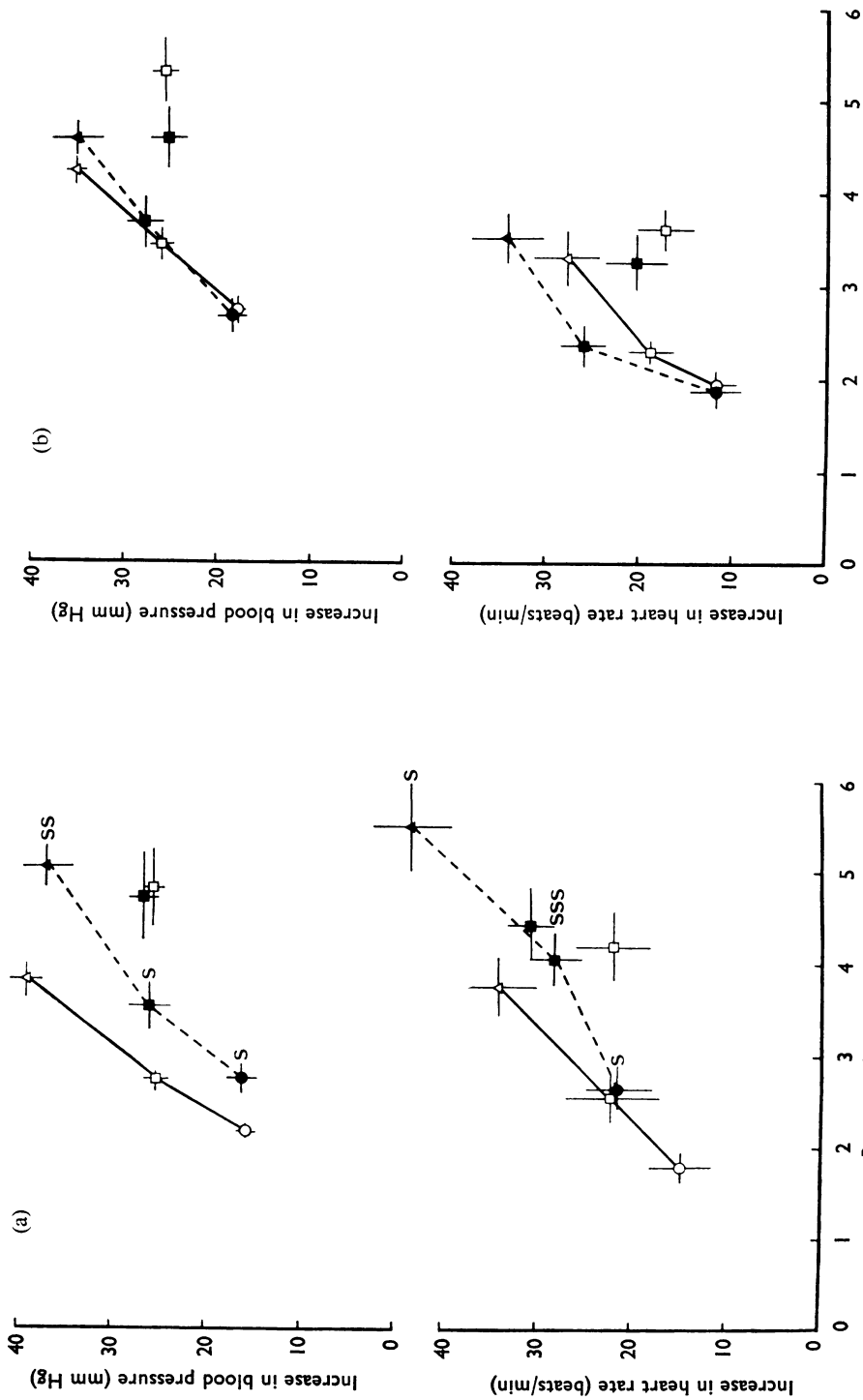


FIG. 1. Effects of progesterone on the size (ordinates) and duration (abscissae) of the cardiovascular responses of the pithed rat to adrenaline (0·025, 0·05, 0·1 μg) (a) and noradrenaline (0·025, 0·05, 0·1 μg) (b). Each low, intermediate and high dose is represented by a circle, square and triangle respectively. The open symbols joined by solid lines represent results from control animals; the closed symbols joined by broken lines represent results from animals pretreated for 14 days with progesterone (20 mg/kg). The points not joined by lines represent responses to adrenaline (0·05 μg) (a) and noradrenaline (0·05 μg) (b) re-established after the injection of pyrogallol (5 mg/kg). Points are the means of between six and nine observations: the horizontal and vertical lines indicate standard errors of the means. $S=0{\cdot}05>P>0{\cdot}01$; $SS=0{\cdot}01>P>$

Effects of progesterone on the pressor and heart rate responses to adrenaline, noradrenaline, tyramine, 5-hydroxytryptamine and stimulation of the whole sympathetic outflow in the pithed rat

In pithed rats pretreated with progesterone the duration of both the blood pressure and heart rate responses to increasing doses of adrenaline (0·025, 0·05, 0·1 μg) were significantly increased (Fig. 1a). In contrast, the magnitudes of the pressor responses remained closely similar in both groups and the heart rate responses, although increased, were not significantly different from their equivalent controls (Fig. 1a).

The cardiovascular responses to noradrenaline (0·025, 0·05, 0·1 μg) (Fig. 1b) and tyramine (2·5, 5, 10 μg) (Fig. 2a) elicited in progesterone treated animals did not differ significantly in magnitude or duration from the equivalent responses to control animals.

5-Hydroxytryptamine (1, 2, 4 μg) gave inconsistent and small effects on heart rate and only the results with the pressor responses are presented. These were closely

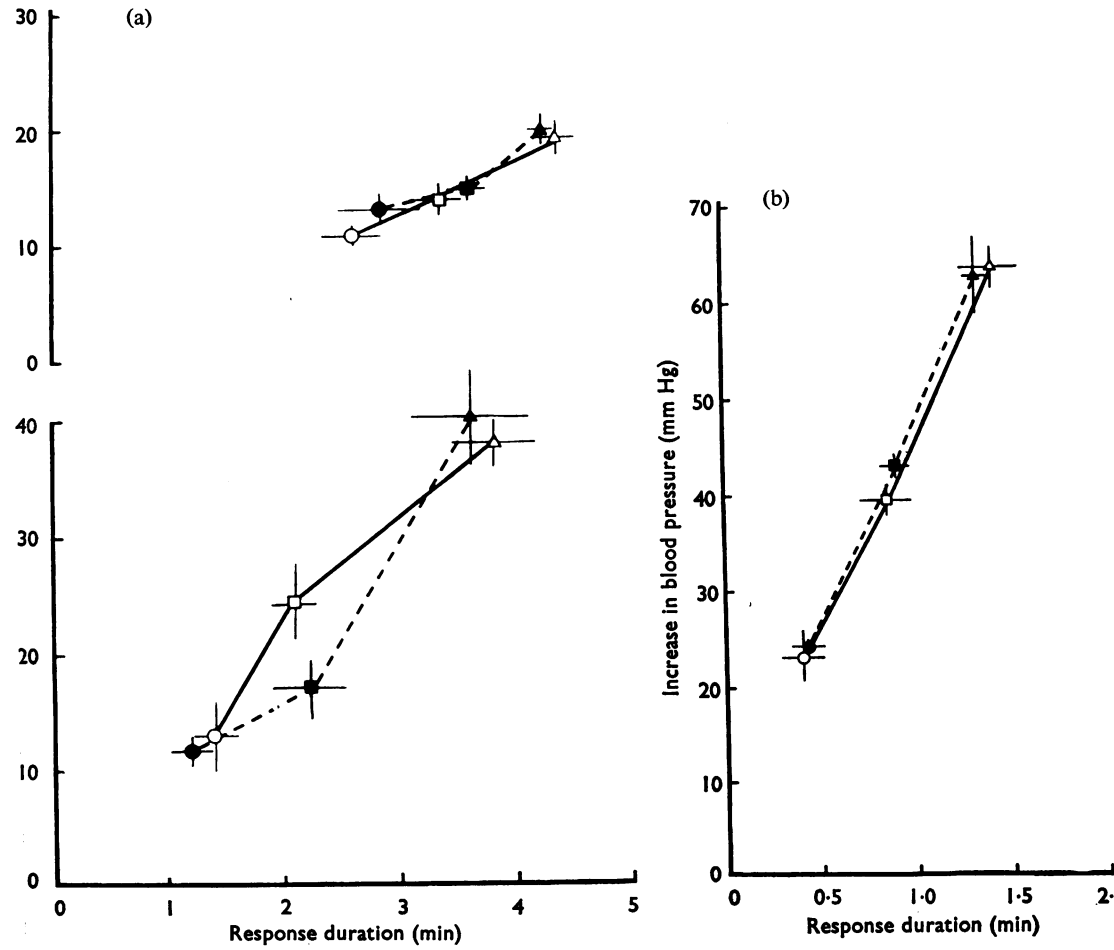


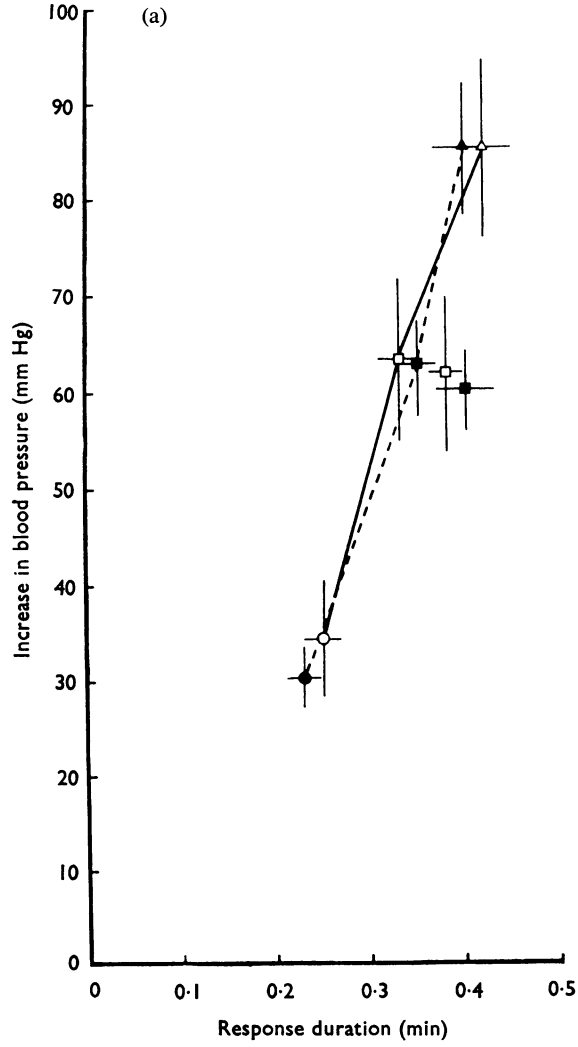
FIG. 2. Effects of progesterone on the size (ordinates) and duration (abscissae) of the cardiovascular responses of the pithed rat to tyramine (2·5, 5, 10 μg) (a) and 5-hydroxytryptamine (1, 2, 4 μg) (b). Other details as Fig. 1.

similar in both magnitude and duration in the control and progesterone pretreated groups (Fig. 2b).

The initial transient rise in blood pressure as a result of stimulating the spinal sympathetic outflow was little changed in either magnitude or duration by pretreatment of rats with progesterone (Fig. 3a). In contrast, the secondary rise in blood pressure and the heart rate response which coincided with it, were significantly increased in duration. As with adrenaline, the magnitudes of the cardiovascular responses were not significantly different in the two groups (Fig. 3b).

Effects of pyrogallol on the cardiovascular responses to adrenaline, noradrenaline and stimulation of the spinal sympathetic outflow in control and progesterone pretreated rats

Pyrogallol (5 mg/kg) was injected intravenously after the control dose-response curves to adrenaline, noradrenaline and sympathetic stimulation had been estab-



(3a)

Legend to 3a on page 193.

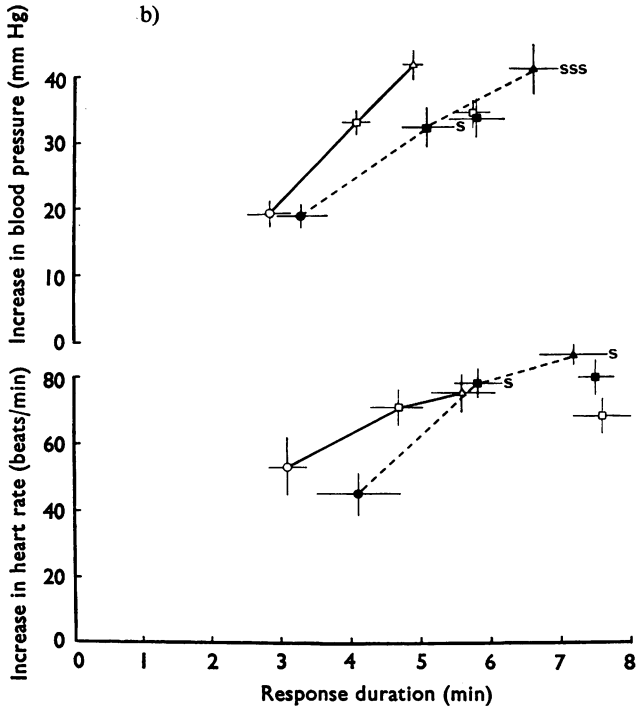


FIG. 3. Effects of progesterone on the size (ordinates) and duration (abscissae) of the cardiovascular responses of the pithed rat to the first (a) and second phase (b) of the response to stimulation of the sympathetic outflow from the spinal cord (1, 3, 6 Hz). The points not joined by lines represent responses to sympathetic stimulation (3 Hz) re-established after the injection of pyrogallol (5 mg/kg). Other details as in Fig. 1.

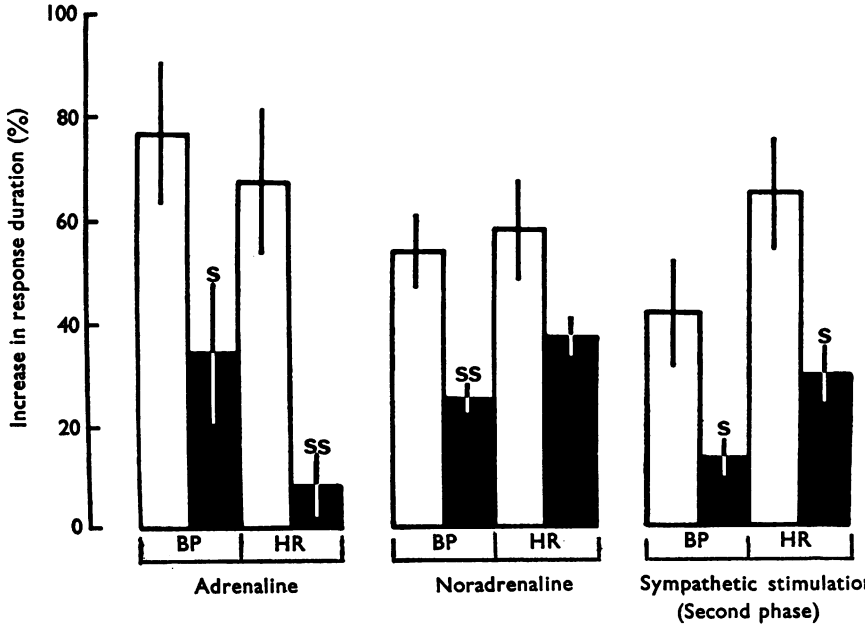


FIG. 4. Percentage increases in duration of the blood pressure (BP) and heart rate (HR) responses to adrenaline (0·05 μg), noradrenaline (0·05 μg) and sympathetic stimulation (3 Hz) due to pyrogallol (5 mg/kg). The open columns represent results from control animals, and the closed columns represent those from progesterone treated animals and are the means of six to nine observations with standard errors. $S=0{\cdot}05>P>0{\cdot}01$-; $SS=0{\cdot}01>P>0{\cdot}001$, refer to the significance of the difference between corresponding mean values.

lished. Approximately 30 min later, responses to single doses of adrenaline (0·05 μg), noradrenaline (0·05 μg) and sympathetic stimulation (3 Hz) were re-established. In each case there was a marked increase in the duration of the blood pressure and heart rate responses without a significant effect on the magnitude (Figs. 1 and 3). When expressed as percentage increases over the control values, the cardiovascular response durations after pyrogallol were consistently greater in control preparations than in the progesterone pretreated animals (Fig. 4).

Effects of progesterone on the urinary excretion of radioactivity after an intraperitoneal injection of 12·5 μCi of (±) isoprenaline-7-^{3}H

The results are shown in Table 1. No significant difference could be demonstrated between control and progesterone pretreated animals with respect to the total 7·25 h urine volume or the percentage of the administered dose excreted. Further, the proportion of the excreted radioactivity which could be ascribed to catechol and non-catechol metabolites of (±) isoprenaline-7-^{3}H was closely similar in the two groups.

Discussion

Gillespie & Muir (1967) suggested that the initial transient rise in blood pressure resulting from stimulation of the whole spinal sympathetic outflow was due to noradrenaline release from the sympathetic nerves, and the secondary prolonged rise was due to catecholamine release from the adrenal medullae. The present experiments show a relatively specific effect of progesterone in increasing the duration, but not the magnitude, of the pressor and heart rate responses to adrenaline and of that phase of the response to sympathetic stimulation attributable to adrenal medullary catecholamine release (Figs. 1a and 3b). That these are affected similarly is perhaps not surprising since rat adrenal glands contain approximately 90% adrenaline (Burn, Hutcheon & Parker, 1950 ; Shepherd & West, 1951), and would therefore be expected to release mainly adrenaline on stimulation.

In contrast, the cardiovascular effects of noradrenaline administered exogenously (Fig. 1b), or released endogenously by tyramine or nerve stimulation (Figs. 2a and 3a), and 5-hydroxytryptamine (Fig. 2b), did not differ significantly in the two groups. It is unlikely therefore, that progesterone produces impairment of the tissue uptake inactivation processes in the pithed rat for either noradrenaline (Muscholl, 1961 ; Bonaccorsi & Garattini, 1966 ; Pals, Fulton & Masucci, 1968), or 5-hydroxytrypta-

TABLE 1. *Urinary excretion of radioactivity after an intraperitoneal injection of* 12·5 μCi (1·09 μg) *of* (±)-*isoprenaline-7-*^{3}*H*

	n	Total 7·25 h urine volume (ml)	Total excreted radioactivity (% of administered dose)	Proportion of excreted radioactivity present as: non-catechol metabolites (%)	Proportion of excreted radioactivity present as: catechol metabolites (%)
Control group (ethyl oleate pretreated)	5	10·7±0·8	26·0±3·8	97·2±2·6	6·0±0·7
Test group (progesterone 20 mg/kg daily for 14 days)	5	12·1±0·7	27·8±5·3	96·9±3·3	6·7±0·6

mine (Fozard, 1969). Further, the release processes for noradrenaline either by tyramine or sympathetic stimulation were obviously unimpaired.

Southgate, Grant, Pollard, Pryse-Davies & Sandler (1968), and Grant & Pryse-Davies (1968), demonstrated a dramatic increase in monoamineoxidase activity of the progesterone dominated rat and human endometrium, and speculated that similar changes might occur in other tissues. The present experiments suggest that for the rat cardiovascular system at least, this is unlikely. Changes in responses to tyramine are a sensitive indicator of changes in monoamineoxidase activity in the pithed rat (Clarke & Leach, 1968), yet responses to tyramine remained closely similar in the two groups (Fig. 2a).

Kalsner (1969a) suggested that certain steroids, including progesterone, potentiated catecholamine cardiovascular responses on the rabbit aortic strip by inhibiting a major inactivation mechanism, probably the enzyme catechol-O-methyl transferase (COMT). O-methylation is the principal route for the inactivation of circulating catecholamines in the intact rat (Axelrod, Inscoe, Senoh & Witkop, 1958), and the following evidence suggests a similar explanation may apply to the present results.

First, the duration, but not the magnitude of the response to adrenaline and the second phase of that to sympathetic stimulation was enhanced by progesterone (Figs. 1a and 3b). This is in good agreement both with reports in the literature (Wylie, Archer & Arnold, 1960 ; Murnaghan & Mazurkiewicz, 1963 ; Izquierdo & Kaumann, 1963 ; Ross, 1963 ; Trendelenburg, 1965), and the present observations with pyrogallol (Figs. 1a and 1b, 3a and 3b), that inhibitors of COMT prolong *in vivo* responses to catecholamines without affecting their magnitudes.

Second, in the present experiments, progesterone induces prolongation of those cardiovascular responses mediated by adrenaline, but not those mediated by noradrenaline. It is known that a greater proportion of adrenaline than noradrenaline is O-methylated following injection of tritiated amine in cats and dogs (Axelrod, Weil-Malherbe & Tomchick, 1959 ; Whitby, Axelrod & Weil-Malherbe, 1961). Further, COMT inhibitors in general enhance responses to adrenaline more than noradrenaline both *in vivo* (Wylie *et al.*, 1960 ; Ross, 1963 ; Trendelenburg, 1965), and *in vitro* (Kalsner, 1969a and b). Indeed, with the appropriate dose of inhibitor, increased responsiveness to adrenaline can occur without a concomitant increase in the responsiveness to noradrenaline (Wylie *et al.*, 1960 ; Ryall, 1961).

Finally, the extent of the prolongation of the pressor and heart rate responses to adrenaline, noradrenaline and sympathetic stimulation by pyrogallol was consistently less in progesterone treated animals than in the equivalent controls (Fig. 4), suggesting a similar basic mechanism of action. Kalsner (1969a) drew similar conclusions from his experiments on the rabbit aortic strip, when pretreatment with progesterone prevented the usual enhancement of amine responses by COMT inhibitors.

In contrast, the evidence from the experiments with (±) isoprenaline-7-^{3}H does not support the hypothesis that progesterone is inhibiting O-methylation, since the ratio of catechol to non-catechol metabolites excreted in the urine was closely similar in both the test and the control groups (Table 1). In confirmation of the results of Carlsson & Waldeck (1963), the radioactivity excreted was almost exclusively in the non-catechol form. This is probably the result of absorption into the portal hepatic circulation in consequence of the intraperitoneal route of administration.

Although these observations indicate no difference between the ability of animals from each group to O-methylate (±) isoprenaline-7-^{3}H, it must be conceded that only hepatic COMT activity may have been monitored. The possibility cannot be excluded that progesterone is effecting a local decrease in COMT activity in the tissues of the cardiovascular system which would be too small to influence the overall excretion pattern of metabolites, but would be sufficient to prolong responses to adrenaline.

These results may have some relevance to the prophylactic effect of progesterone in the treatment of migraine. The migraine syndrome includes a phase of painful dilatation of extracranial blood vessels which can be relieved by drug induced vasoconstriction (Wolff, 1963). It is tempting to speculate that the beneficial effects of progesterone might be related to the production in the cardiovascular system of a milieu conducive to the persistence of vasoconstrictor amines (Kalsner, 1969a), particularly adrenaline (present experiments), which are inactivated by COMT.

This work was supported in part by a grant from the Migraine Trust.

REFERENCES

AXELROD, J., INSCOE, J. K., SENOH, S. & WITKOP, B. (1958). O-methylation, the principal pathway for the metabolism of epinephrine and norepinephrine in the rat. *Biochem. Biophys. Acta*, **27**, 210–211.

AXELROD, J., WEIL-MALHERBE, H. & TOMCHICK, R. (1959). The physiological disposition of H^{3}-epinephrine and its metabolite metanephrine. *J. Pharmac. exp. Ther.*, **127**, 251–253.

BONACCORSI, A. & GARATTINI, S. (1966). Effect of desipramine on directly or indirectly elicited catecholamine pressor responses in rats. *J. Pharm. Pharmac.*, **18**, 443–448.

BURN, J. H., HUTCHEON, D. E. & PARKER, R. H. O. (1950). Adrenaline and noradrenaline in the suprarenal medulla after insulin. *Br. J. Pharmac. Chemother.*, **5**, 417–423.

CARLSSON, A. & WALDECK, B. (1963). On the role of liver catechol O-methyl transferase in the metabolism of circulating catecholamines. *Acta pharmac. tox.*, **20**, 47–55.

CLARKE, D. E. & LEACH, G. D. H. (1968). The influence of bretylium on the interactions of infused sympathomimetic amines and tyramine in the reserpine-treated pithed rat. *Br. J. Pharmac. Chemother.*, **32**, 392–401.

FOSTER, R. W. (1968). A correlation between inhibition of the uptake of ^{3}H from (±)-^{3}H-noradrenaline and potentiation of the responses to (−)-noradrenaline in the guinea-pig isolated trachea. *Br. J. Pharmac. Chemother.*, **33**, 357–367.

FOSTER, R. W. (1969). An uptake of radioactivity from (±)-^{3}H-isoprenaline and its inhibition by drugs which potentiate the responses to (−) isoprenaline in the guinea-pig isolated trachea. *Br. J. Pharmac.*, **35**, 418–417.

FOZARD, J. R. (1969). Drug induced changes in the distribution of ^{14}C 5-HT in the pithed reserpinised rat. *Europ. J. Pharmac.*, **7**, 248–257.

FOZARD, J. R. & LEACH, G. D. H. (1968). The hypotensive action of 5-hydroxytryptamine (5-HT) in anaesthetised and pithed rats. *Europ. J. Pharmac.*, **2**, 239–249.

FOZARD, J. R. & SCHNIEDEN, H. (1970). Steroidal hormones and vascular reactivity in the rabbit. In: *Background to Migraine*, **III**, Third International Migraine Symposium, ed. Cochrane, A. L. A. 136–149. London: Heinemann.

GILLESPIE, J. S. & MUIR, T. C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. *Br. J. Pharmac. Chemother.*, **30**, 74–87.

GRANT, E. C. G. & PRYSE-DAVIES, J. (1968). Effect of oral contraceptives on depressive mood changes and on endometrial monoamineoxidase and phosphatases. *Br. med. J.*, **3**, 777–780.

GREENE, R. (1963). Migraine—the menstrual aspect. *J. Coll. gen. Pract.*, **6**, Supp: 4, 15–17.

GREENE, R. (1967). Menstrual headache. *Res. clin. Stud. Headache.* **1**, 62–73. Basel/New York: Karger.

GREENE, R. & DALTON, K. D. (1953). The pre-menstrual syndrome. *Br. med. J.*, **1**, 1007–1014.

IZQUIERDO, J. A. & KAUMANN, A. J. (1963). Effects of pyrogallol on the duration of the cardiovascular action of catecholamines. *Arch. int. Pharmacodyn. Ther.*, **144**, 437–445.

KALSNER, S. (1969a). Steroid potentiation of responses to sympathomimetic amines in aortic strips. *Br. J. Pharmac.*, **36**, 583–593.

KALSNER, S. (1969b). Mechanism of hydrocortisone potentiation of responses to epinephrine and norepinephrine in rabbit aorta. *Circulation Res.*, **24**, 383–396.

KOPIN, I. J., AXELROD, J. & GORDON, E. (1961). The metabolic fate of H^{3}-epinephrine and C^{14}-metanephrine in the rat. *J. biol. Chem.*, **236**, 2109–2113.

MURNAGHAN, M. F. & MAZURKIEWICZ, I. M. (1963). Some pharmacological properties of 4-methyltropolone. *Rev. canad. biol.*, **22**, 99–102.

MUSCHOLL, E. (1961). Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. *Br. J. Pharmac. Chemother.*, **16**, 352–359.

PALS, D. T., FULTON, R. W. & MASUCCI, F. D. (1968). Angiotensin, cocaine and desipramine: Comparison of effects on blood pressure responses to norepinephrine, tyramine and phenylephrine in the pithed rat. *J. Pharmac. exp. Ther.*, **162**, 85–91.

ROSS, S. B. (1963). *In vivo* inactivation of catecholamines in mice. *Acta pharmac. tox.*, **20**, 267–273.

RYALL, R. W. (1961). Effects of cocaine and antidepressant drugs on the nictitating membrane of the cat. *Br. J. Pharmac. Chemother.*, **17**, 339–357.

SHEPHERD, D. M. & WEST, G. B. (1951). Noradrenaline and the suprarenal medulla. *Br. J. Pharmac. Chemother.*, **6**, 665–674.

SINGH, I., SINGH, I. & SINGH, D. (1947). Progesterone in the treatment of migraine. *Lancet*, **1** 745–747.

SOUTHGATE, J., GRANT, E. C. G., POLLARD, W., PRYSE-DAVIES, J. & SANDLER, M. (1968). Cyclical variation in endometrial monoamineoxidase: correlation of histochemical and biochemical assays. *Biochem. Pharmacol.*, **17**, 721–726.

TRENDELENBERG, U. (1965). Supersensitivity by cocaine to dextrorotatory isomers of norepinephrine and epinephrine. *J. pharmac. exp. Ther.*, **148**, 329–338.

WEIL-MALHERBE, H. & BONE, A. D. (1952). The chemical estimation of adrenaline-like substances in blood. *Biochem. J.*, **51**, 311–318.

WHITBY, L. G., AXELROD, J. & WEIL-MALHERBE, H. (1961). The fate of H^3-norepinephrine in animals. *J. pharmac. exp. Ther.*, **132**, 193–201.

WOLFF, H. G. (1963). *Headache and Other Head Pain.* New York: Oxford University Press.

WYLIE, D. W., ARCHER, S. & ARNOLD, A. (1960). Augmentation of pharmacological properties of catecholamines by O-methyl transferase inhibitors. *J. Pharmac. exp. Ther.*, **130**, 239–244.

(*Received August* 18, 1970)