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THE INFLUENCE OF PHENOXYBENZAMINE ON THE STORAGE OF NORADRENALINE IN RAT AND CAT TISSUES

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In the cat, phenoxybenzamine prevented the uptake of noradrenaline by the kidney but not by the uterus; dichloroisoprenaline prevented the uptake of noradrenaline by the uterus but not by the kidney. In the rat, a single dose of phenoxybenzamine prevented the uptake of noradrenaline by the heart, spleen and uterus and reduced the uptake by the duodenum. Prolonged treatment with phenoxybenzamine prevented the uptake by the heart and uterus but not by the spleen or duodenum. Both phenoxybenzamine and dichloroisoprenaline lowered the noradrenaline content of some tissues of the rat. Phenoxybenzamine did not antagonize the responses to noradrenaline of the nonpregnant cat uterus *in situ*, nor the responses of rat isolated ventricles, uterus or duodenum. It is concluded that the way in which these noradrenaline antagonists affect the storage sites for noradrenaline varies between organs. The effects of the drugs on the receptors for noradrenaline are not related to their effects on storage sites.

Phenoxybenzamine prevents some actions of noradrenaline by occupying receptor sites (Nickerson & Nomaguchi, 1951). It also modifies the concentration of noradrenaline in tissues and blood. Shapiro (1958) showed that the noradrenaline contents of rat heart and spleen were lowered, and Millar, Keener & Benfey (1959) found that plasma levels of noradrenaline were increased after giving phenoxybenzamine. Some time ago, Cannon & Bacq (1931) showed that a small dose of ergotamine increased the amount of sympathin reaching the heart when the sympathetic nerves to the hind-limb were stimulated. Later, Brown & Gillespie (1957) found that phenoxybenzamine increased the outflow of noradrenaline from the spleen of the cat when the splenic nerves were stimulated. Although they suggested that by occupying the receptor sites phenoxybenzamine was preventing the destruction of noradrenaline, an alternative explanation for their results is that phenoxybenzamine may hinder the entry of noradrenaline into the tissue store.

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In the present study we have investigated the actions of phenoxybenzamine on noradrenaline stores and on noradrenaline receptors. Another antagonist, dichloroisoprenaline, which prevents responses to noradrenaline other than those prevented by phenoxybenzamine, has been tested for its effects on storage sites and on receptors for noradrenaline. A preliminary account of this work has been published (Farrant, Harvey & Pennefather, 1961).

METHODS

Spinal cats were prepared by Dale's method, as described by Burn (1952). Blood pressure was recorded from one carotid artery and a polyethylene tube was inserted into the other to allow the withdrawal of blood samples. The increase in the noradrenaline content of the kidney and uterus after infusion of noradrenaline was studied by the method of Pennefather & Rand (1960). One kidney and one horn of the uterus were removed and 20 min later noradrenaline was infused (1 mg in 14.6 ml. of solution during 40 min). The remaining kidney and uterine horn were removed 20 min after the infusion had ceased. Blood samples were taken when the organs were removed. Only nonpregnant cats were used.

The effect of infusions of noradrenaline on the noradrenaline content of rat tissues was studied in experiments similar to those of Muscholl (1961a, b). The brain and spinal cord of rats were destroyed by pithing and the blood pressure was recorded from one carotid artery; 20 min later a blood sample was taken and noradrenaline infused (80 μg in 0.8 ml. of solution during 20 min). The heart, uterus, spleen and duodenum were removed 20 min after the infusion had ended and another sample of blood was taken.

The effects of phenoxybenzamine and dichloroisoprenaline on the uptake of noradrenaline were tested by injecting the drug intravenously immediately after the animals had been made spinal or pithed. Phenoxybenzamine was given in a dose of 10 mg/kg to the cat and 6 mg/kg to the rat; dichloroisoprenaline was given in a dose of 10 mg/kg into cats.

The effects of daily intraperitoneal injections of phenoxybenzamine (6 mg/kg/day for 7 days) on the noradrenaline content of rat tissues and on the uptake of infused noradrenaline by these tissues were also studied. Ten rats were pithed 24 hr after the last dose of phenoxybenzamine and the organs removed 1 hr later. In another ten rats, 20 min after pithing the infusion of noradrenaline was started, and 20 min after the completion of this infusion the organs were removed. The effects of seven daily intraperitoneal injections of dichloroisoprenaline (10 mg/kg/day) on the noradrenaline content of rat tissues were studied in ten rats.

Extraction and assay of noradrenaline. Blood samples were collected into centrifuge tubes containing 0.05 ml. of heparin (5000 U/ml.) and immediately centrifuged (3000 rev/min for 15 min). The plasma was drawn off with a syringe and stored at $-10°$ C. The tissues were extracted as described by Burn & Rand (1959).

Extracts were assayed on the blood pressure of the atropinized pithed rat for activity in terms of noradrenaline. When assaying extracts of the kidney it was first necessary to give repeated doses until the effects of the "slow pressor component" were eliminated (Pennefather & Rand, 1960).

Effects of phenoxybenzamine and dichloroisoprenaline on responses to noradrenaline and adrenaline. Movements of one horn of the cat uterus *in situ* were recorded with a frontal writing lever. Cats were made spinal but were not eviscerated, and the blood pressure was recorded from one carotid artery. Drugs were injected intravenously.

Isolated tissues from the rat were suspended in Krebs bicarbonate solution bubbled with air and maintained at 32° C. Contractions of the right ventricle were elicited by stimulating through platinum wires using rectangular wave pulses of 0.3 msec duration at 2 V every 12 sec (Stewart, 1958). Regular contractions of the uterus were elicited by the application of a 50 cycles/sec a.c. stimulation of 4 to 12 V for 10 to 20 sec every 2.5 min (Harvey & Pennefather, 1962). Duodenal segments were stimulated with 50 cycles/sec a.c. at 4 to 12 V and applied for 5 sec every 1.5 min. The electrical a.c. stimulation was given through silver electrodes attached at each end of the tissue.

Drugs. Amounts of phenoxybenzamine hydrochloride (Smith, Kline & French) and dichloroisoprenaline hydrochloride (Eli Lilly) are given in terms of their salts. Amounts of (−)-noradrenaline (Hoechst) and (−)-adrenaline (B.D.H.) are given in terms of the bases.

RESULTS

Uptake of noradrenaline by cat tissues. Infusions of noradrenaline increase the noradrenaline content of cat kidney and uterus (Pennefather & Rand, 1960). However, in six cats injected with phenoxybenzamine the uptake of noradrenaline by the kidney was prevented, but the uptake by the uterus was not affected (Fig. 1). On the other hand, in six cats injected with dichloroisoprenaline, the uptake of noradrenaline by the uterus was prevented but the uptake by the kidney was not altered (Fig. 2).

Responses of the cat uterus. In three cats phenoxybenzamine (10 mg/kg) did not modify the relaxation of the uterus caused by noradrenaline (5 μg) or by adrenaline (1 μg). In contrast, dichloroisoprenaline (10 mg/kg) completely abolished these inhibitory responses, which result confirms the findings of Powell & Slater (1958).

Noradrenaline content of rat tissues. The mean noradrenaline contents of tissues from rats that had been given either one injection or seven daily injections of phenoxybenzamine or seven daily injections of dichloroisoprenaline are shown in Table 1. The injection of a single dose of phenoxybenzamine reduced the noradrenaline content of the spleen and duodenum but not that of the heart or uterus. Prolonged treatment lowered the noradrenaline content of the spleen, duodenum

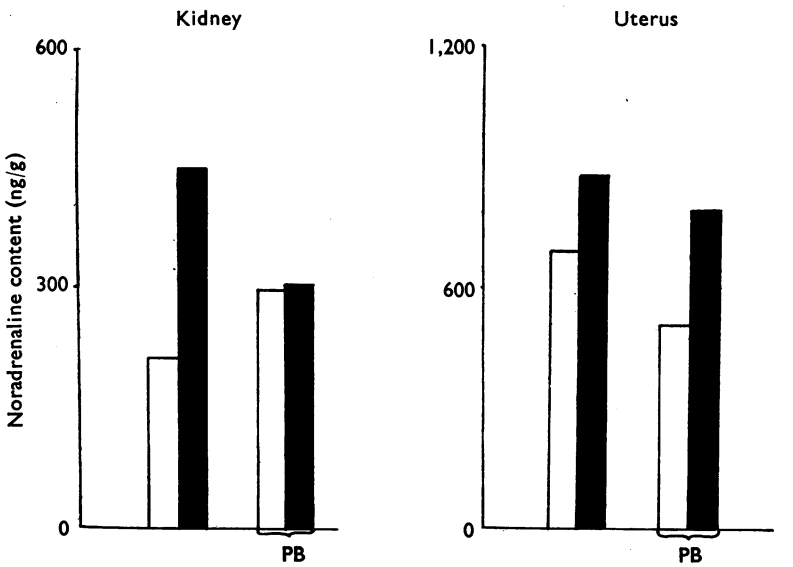

Fig. 1. Effect of phenoxybenzamine (PB, 10 mg/kg) on the increase in noradrenaline content (ng/g) of the cat kidney and uterus after an infusion of noradrenaline (1 mg during 40 min). There were six cats in each group. Empty columns before and filled columns after infusion of noradrenaline.

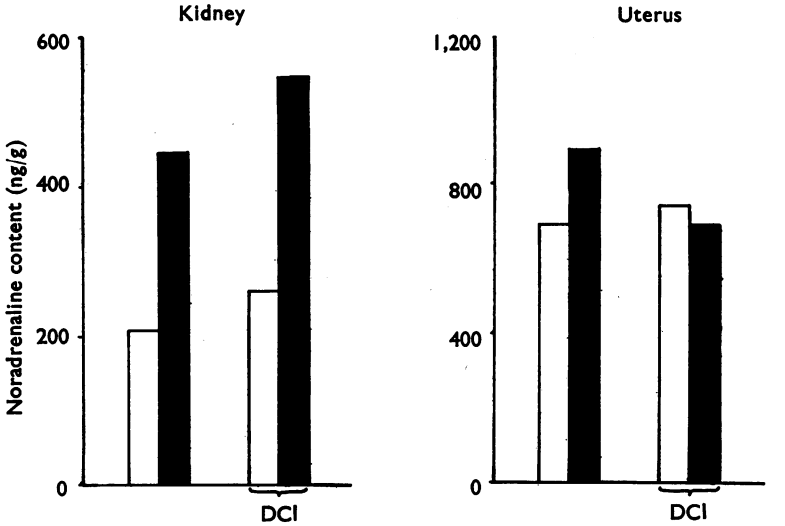

Fig. 2. Effect of dichloroisoprenaline (DCI, 10 mg/kg) on the increase in noradrenaline content (ng/g) of cat kidney and uterus after an infusion of noradrenaline (1 mg during 40 min). There were six cats in each group. Empty columns before and filled columns after infusion of noradrenaline.

TABLE 1

MEAN NORADRENALINE CONTENTS OF TISSUES FROM UNTREATED RATS AND FROM RATS INJECTED WITH PHENOXYBENZAMINE OR DICHLOROISOPRENALINE

There were ten rats in each group and the means and standard errors are given

Treatment	Noradrenaline content in Heart (ng/g)	Spleen (ng/tissue)	Duodenum (ng/g)	Uterus (ng/g)
Untreated	520± 60	315±33	296±38	187±21
Single injection of phenoxybenzamine (6 mg/kg)	550±150	214±48	233±16	187±70
Seven daily injections of phenoxybenzamine (6 mg/kg/day)	295± 30	213±26	217±41	208±21
Seven daily injections of dichloroisoprenaline (10 mg/kg/day)	333± 44	62±15	58±15	68±17

and heart, but not that of the uterus. Seven daily injections of dichloroisoprenaline reduced the noradrenaline content of all four tissues. Dichloroisoprenaline reduced the noradrenaline content of the spleen and duodenum to a greater extent than did phenoxybenzamine; the content in the heart was reduced to the same extent by both drugs.

Uptake of noradrenaline by rat tissues. Infusions of noradrenaline increased the mean noradrenaline content of the heart and spleen, confirming the results of Muscholl (1961a, b); the content of the uterus was also increased but uptake by

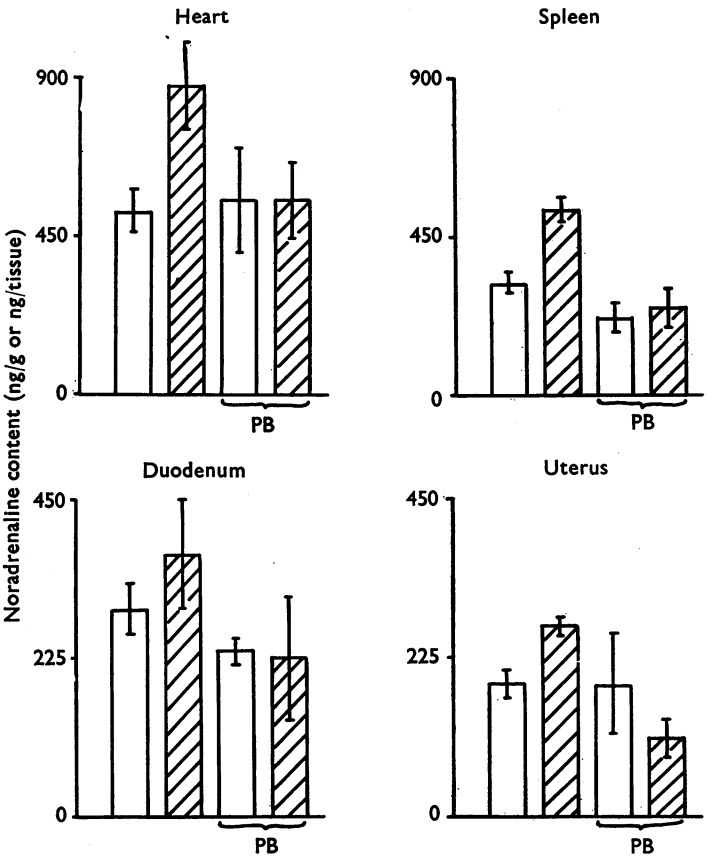

Fig. 3. Effect of an infusion of noradrenaline (80 μg during 20 min) on the mean noradrenaline contents (ng/g for heart, duodenum and uterus, and ng/tissue for spleen) of tissues from untreated rats and from rats injected with phenoxybenzamine (6 mg/kg, PB). There were six rats in each group. The vertical bars represent the standard errors of the means. The uptake of noradrenaline is prevented by phenoxybenzamine. Empty columns before and hatched columns after infusion of noradrenaline.

the duodenum was less marked (Fig. 3). After a single injection of phenoxybenzamine the uptake of infused noradrenaline by the heart, spleen and uterus was prevented, and the uptake by the duodenum reduced. After prolonged treatment with phenoxybenzamine the uptake by the heart and uterus was prevented, the uptake by the spleen was reduced, whilst the uptake by the duodenum was unaltered (Fig. 4).

Responses of rat tissues. The force of contraction of the electrically stimulated right ventricle was increased when noradrenaline (1 μg/ml.) was added to the bath. Phenoxybenzamine potentiated this action of noradrenaline (Fig. 5). When the concentration of phenoxybenzamine was increased five-times it produced a long-lasting increase in the force of contraction, but even then the responses to noradrenaline were not reduced. The electrically induced contractions of the uterus

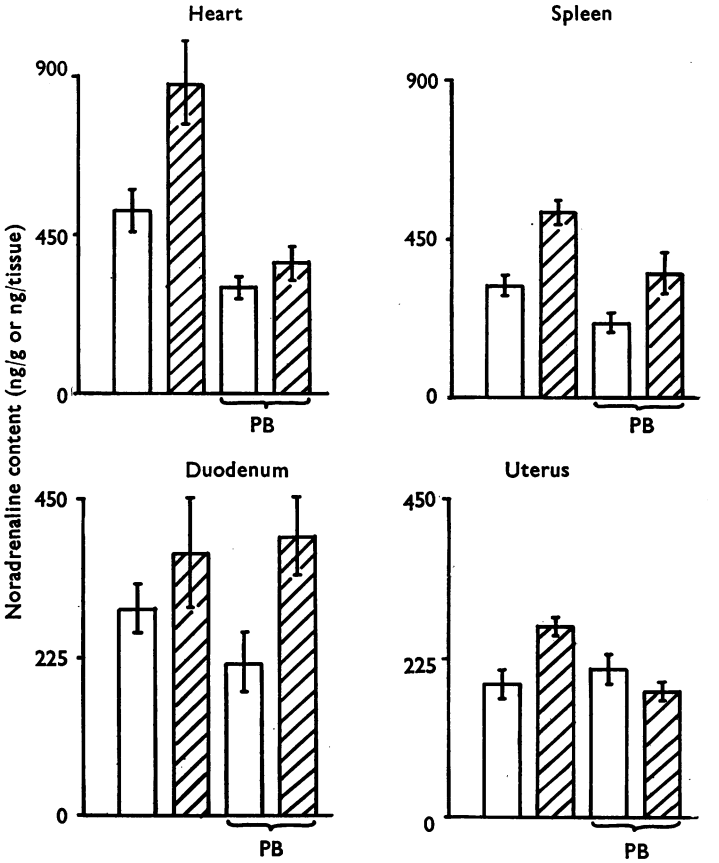

Fig. 4. Effect of an infusion of noradrenaline (80 μg during 20 min) on the mean noradrenaline contents (ng/g for heart, duodenum and uterus and ng/tissue for spleen) of tissues from untreated rats and from rats given seven daily injections of phenoxybenzamine (6 mg/kg/day, PB). There were ten rats in each group. The vertical bars represent standard errors of the means. Prolonged treatment with phenoxybenzamine prevented the uptake of noradrenaline by the heart and uterus. Empty columns before and hatched columns after infusion of noradrenaline.

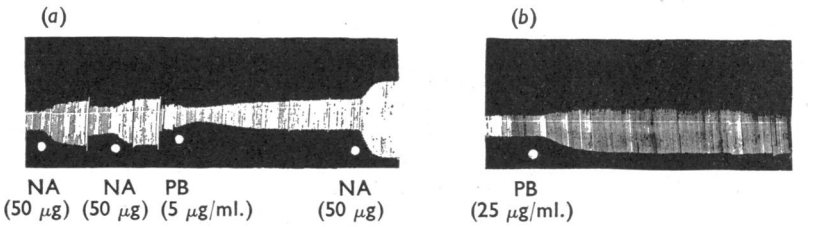

Fig. 5. (*a*) Shows the effect of 5 μg/ml. of phenoxybenzamine (PB) on the increase in the force of contraction of an electrically stimulated rat ventricle produced by noradrenaline (NA, 1·μg/ml.). The response to noradrenaline was not blocked by phenoxybenzamine. In (*b*), a concentration of 25 μg/ml. of phenoxybenzamine itself increased the force of contraction.

were inhibited by noradrenaline (1 μg/ml.) and by adrenaline (0.2 ng/ml.). Phenoxybenzamine (1 μg/ml.) enhanced these inhibitory responses. Higher concentrations of phenoxybenzamine (100 μg/ml.) slightly inhibited the contractions but the responses to the catechol amines were neither enhanced nor reduced. The contractions of the electrically stimulated duodenal segments were inhibited by noradrenaline (50 ng/ml.) and by adrenaline (0.2 ng/ml.). Phenoxybenzamine (10 μg/ml.) did not affect these inhibitory responses.

Plasma noradrenaline. The mean noradrenaline level in cat plasma was 27 ng/ml. 20 min after an infusion of noradrenaline (1 mg) the plasma level was increased by 23 ng/ml. in control cats, by 66 ng/ml. in cats injected with phenoxybenzamine, and by 46 ng/ml. in cats injected with dichloroisoprenaline.

In the rat, a single injection of phenoxybenzamine increased the mean plasma noradrenaline level from 15 to 30 ng/ml., and after seven daily doses the mean plasma level was 11 ng/ml. The increase in the plasma noradrenaline from 15 to 50 ng/ml. produced by the infusion of noradrenaline (80 μg) was unaffected by phenoxybenzamine.

DISCUSSION

In a tissue, noradrenaline may be attached at two sites: at the receptor, where its attachment leads to a response, and at another site where it is taken up by the tissue and stored. Our results show that the noradrenaline antagonists phenoxybenzamine and dichloroisoprenaline can act at both sites, but their actions in antagonizing responses to noradrenaline are not related to their actions in preventing the uptake of infused noradrenaline. In the rat heart and uterus, for example, phenoxybenzamine prevented the uptake of infused noradrenaline, but did not antagonize responses to noradrenaline. Thus in the rat heart and uterus phenoxybenzamine acts only at the storage sites, whereas in the spleen it may act at both the storage and the receptor sites. In the uterus of the cat phenoxybenzamine prevents neither the uptake nor the actions of noradrenaline, whereas in the rat uterus the uptake but not the actions of noradrenaline is prevented. Thus the activity of phenoxybenzamine at the store in a tissue cannot be predicted from its activity at the receptor in that tissue.

Since phenoxybenzamine and dichloroisoprenaline prevent the uptake of infused noradrenaline in some but not in all tissues, it appears that the sites of uptake for noradrenaline vary between tissues. In any one tissue, however, there is only one uptake site, which may be blocked either by phenoxybenzamine or by dichloroisoprenaline or by both drugs. Thus the uptake of noradrenaline is prevented in cat kidney by phenoxybenzamine, in cat uterus by dichloroisoprenaline, and in rat heart both by phenoxybenzamine (present results) and by dichloroisoprenaline (Muscholl, 1961a, b). Muscholl also found that dibenamine did not significantly affect the uptake of noradrenaline by this tissue; it may be that in his experiments this drug was injected immediately before the infusion of noradrenaline so that the action at the uptake sites did not have time to develop. If the positive actions of phenoxybenzamine and dichloroisoprenaline at the store were occurring at different sites, then uptake of noradrenaline would be prevented completely only

when both were present. This is in contrast to the receptor sites, for a tissue may contain one or both of the two types of noradrenaline receptors (Ahlquist & Levy, 1959), which are blocked either by phenoxybenzamine or by dichloroisoprenaline but not by both drugs.

In some tissues the noradrenaline antagonists act at both receptor sites and storage sites. In the cat uterus, dichloroisoprenaline prevented the uptake of infused noradrenaline and also antagonized the responses to noradrenaline of this organ, whereas phenoxybenzamine was inactive at both sites. In the cat spleen, the results of Brown & Gillespie (1957) indicate that phenoxybenzamine prevents the uptake of noradrenaline as well as the responses to noradrenaline.

Our results also show that treatment with a single or several doses of phenoxybenzamine or several doses of dichloroisoprenaline reduced the noradrenaline content of some tissues. Shapiro (1958) has previously reported that prolonged treatment with phenoxybenzamine reduces the noradrenaline content of rat heart, spleen and adrenals. This reduction of the tissue content of noradrenaline is not simply a result of the actions of the drugs in preventing the uptake of circulating noradrenaline, since phenoxybenzamine prevented uptake in the rat uterus but did not cause depletion. In all other tissues in which phenoxybenzamine or dichloroisoprenaline prevented uptake, they also lowered the noradrenaline content, perhaps by penetrating to the store and acting directly on the noradrenaline binding sites.

Noradrenaline antagonists may therefore have one or more of three separate actions in any one tissue—they may antagonize the responses to noradrenaline by occupying receptor sites, they may prevent the uptake of infused noradrenaline, and they may lower the noradrenaline content by interfering with the binding of noradrenaline. In contrast to the receptor sites, there is in a tissue only one uptake site, but this site varies between tissues, since the uptake of noradrenaline is not prevented by both drugs in all tissues. The classification of the noradrenaline receptors into α and β types (Ahlquist, 1948) cannot therefore be applied to uptake sites, and the activity of a noradrenaline antagonist at the store cannot be predicted from its activity at the noradrenaline receptor.

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REFERENCES

AHLQUIST, R. P. (1948). A study of the adrenotropic receptors. *Amer. J. Physiol.*, **153**, 586–600.

AHLQUIST, R. P. & LEVY, B. (1959). Adrenergic receptive mechanism of canine ileum. *J. Pharmacol. exp. Ther.*, **127**, 146–149.

BROWN, G. L. & GILLESPIE, J. S. (1957). The output of sympathetic transmitter from the spleen of the cat. *J. Physiol. (Lond.)*, **138**, 81–102.

BURN, J. H. (1952). *Practical Pharmacology*. Oxford: Blackwell.

BURN, J. H. & RAND, M. J. (1959). The cause of the super-sensitivity of smooth muscle to noradrenaline after sympathetic degeneration. *J. Physiol. (Lond.)*, **147**, 135–143.

CANNON, W. B. & BACQ, Z. M. (1931). Studies on the conditions of activity in endocrine organs. XXVI. A hormone produced by sympathetic action on smooth muscle. *Amer. J. Physiol.*, **96**, 392–412.

FARRANT, J., HARVEY, J. A. & PENNEFATHER, J. N. (1961). The influence of phenoxybenzamine on the uptake of noradrenaline by cat and rat tissues. *J. Physiol. (Lond.)*, **159**, 49–50*P*.

HARVEY, J. A. & PENNEFATHER, J. N. (1962). Effect of adrenaline infusions on the catechol amine content of cat and rat tissues. *Brit. J. Pharmacol.*, **18**, 183–189.

MILLAR, R. A., KEENER, E. B. & BENFEY, B. G. (1959). Plasma adrenaline and noradrenaline after phenoxybenzamine administration, and during haemorrhagic hypotension, in normal and adrenalectomized dogs. *Brit. J. Pharmacol.*, **14**, 9–13.

MUSCHOLL, E. (1961a). The effects of drugs on the noradrenaline uptake into the heart. *Biochem. Pharmacol.*, **8**, 65.

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

NICKERSON, M. & NOMAGUCHI, G. M. (1951). Adrenergic blocking action of phenoxyethyl analogues of dibenamine. *J. Pharmacol. exp. Ther.*, **101**, 379–396.

PENNEFATHER, J. N. & RAND, M. J. (1960). Increase in noradrenaline content of tissues after infusion of noradrenaline, dopamine, and l-dopa. *J. Physiol. (Lond.)*, **154**, 277–287.

POWELL, C. E. & SLATER, I. H. (1958). Blocking of inhibitory adrenergic receptors by a dichloro analog of isoproterenol. *J. Pharmacol. exp. Ther.*, **122**, 480–488.

SCHAPIRO, S. (1958). Effect of a catecholamine blocking agent (dibenzyline) on organ content and urine excretion of noradrenaline and adrenaline. *Acta physiol. scand.*, **42**, 371–375.

STEWART, G. A. (1958). The actions of digitalis leaf preparations and of cardiac glycosides on the isolated right ventricle of the guinea-pig. *J. Pharm. Pharmacol.*, **10**, 741–754.