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PERIPHERAL NORADRENALINE AND ADRENERGIC TRANSMISSION IN THE RAT

BY

T. L. B. SPRIGGS*

*From the Department of Pharmacology, School of Pharmacy, Brunswick Square, London W.C.*1

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Burn & Rand (1958, 1960a) put forward the hypothesis (now widely accepted) that reserpine impairs adrenergic function by its action in depleting the stores of the transmitter, noradrenaline. When guanethidine, a potent adrenergic-neurone blocking agent (Maxwell, Mull & Plummer, 1959) was also found to deplete catechol amines from peripheral organs (Sheppard & Zimmerman, 1959), it was suggested (Cass, Kuntzman & Brodie, 1960) that the loss of adrenergic function is attributable to the loss of transmitter substance from the nerve endings. It was later shown that the initial blocking action of guanethidine was not related to the state of depletion of the noradrenaline stores (Cass & Spriggs, 1961 ; Gaffney, Chidsey & Braunwald, 1963). In this paper the effects of guanethidine, bretylium, bethanidine and syrosingopine (an analogue of reserpine) on sympathetic transmission and on noradrenaline stores are examined to test the importance of the noradrenaline store for adrenergic transmission.

METHODS

Male Wistar rats, weighing 150 to 250 g, were used throughout. Guanethidine, bretylium, bethanidine or syrosingopine were injected subcutaneously into the scruff of the neck.

Tissue noradrenaline levels

Six rats from each drug-treated group and six from each control group (saline- or solvent-treated) were killed at various times after injection. The heart and spleen were removed, rinsed in tap water, blotted between filter papers, weighed and stored at −10° C. Catechol amines were extracted from the tissues and estimated spectrophotofluorimetrically (in terms of noradrenaline) by the method of Shore & Olin (1958), as modified by Cass & Spriggs (1961) and Callingham & Cass (1963).

Measurement of adrenergic transmission

Rats were anaesthetized with urethane (1.5 g/kg, intraperitoneally) and a tracheal cannula was inserted. Drugs were administered into a femoral vein through a polyethylene cannula and the blood pressure was recorded from a cannula in the left carotid artery using a Condon manometer. In each rat, adrenergic transmission was assessed by one of the following two methods.

Response to physostigmine. The maximal rise in blood pressure induced by an intravenous injection of 20 μg of physostigmine was measured. This is a central action of physostigmine which

* Present address: Departments of Anatomy and Pharmacology, University College of South Wales and Monmouthshire, Newport Road, Cardiff.

results in a peripheral adrenergic discharge; the adrenal gland contributes little to the response (Varagić, 1955; Lešić & Varagić, 1961). Successive responses to physostigmine were consistent, provided a 30 min interval was allowed between doses (Cass & Spriggs, 1961).

Contraction of the inferior eyelid. Preganglionic stimulation of the right superior cervical sympathetic chain elicited retraction of the right inferior eyelid (Gertner, 1956) which was recorded by means of a thread tied through the eyelid and attached vertically downwards to a light lever recording on a lightly smoked drum. The lever gave a magnification of × 40. The head of the rat was immobilized in a special clamp. Supramaximal electrical stimulation with a frequency of 20 shocks/sec and a pulse width of 0.5 msec was applied through bipolar platinum-hook electrodes from a Palmer CVP, H45 rectangular-wave stimulator. The duration of stimulation was 10 sec.

Noradrenaline infusions

Estimation of noradrenaline uptake into tissue. Rats were anaesthetized with urethane (1.5 g/kg) and cannulae were tied into the trachea and one femoral vein. Noradrenaline was infused

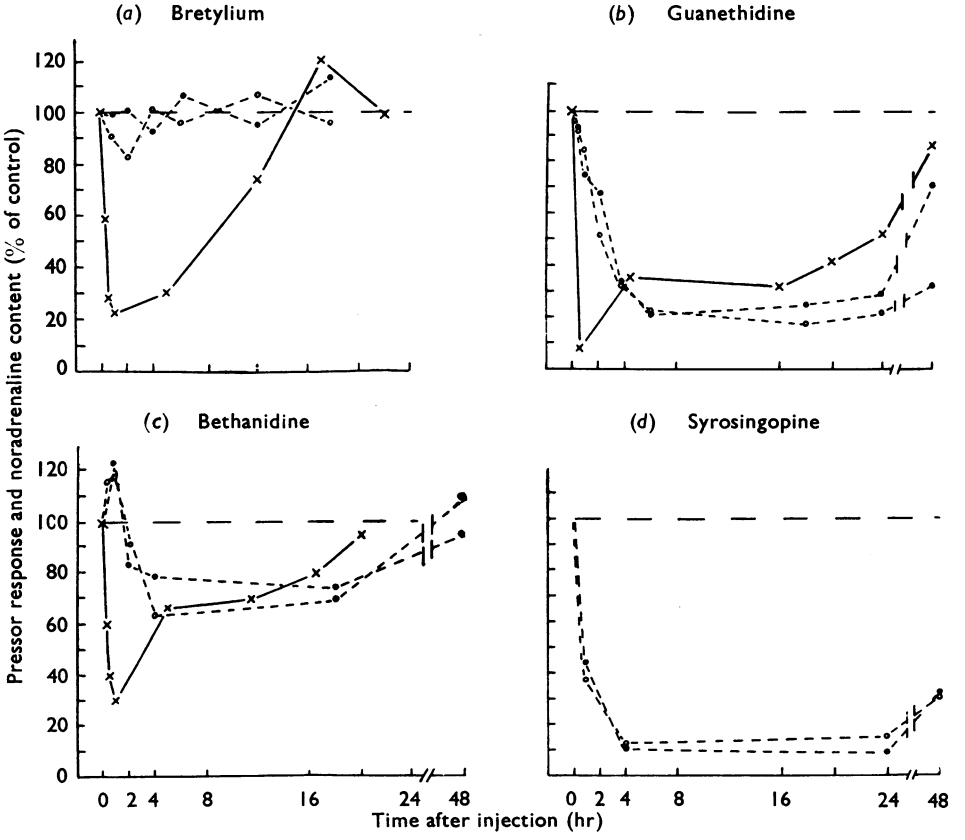

Fig. 1. Sympathetic transmission (×——×; as measured by the pressor response to physostigmine) and noradrenaline content of heart (●——●) and spleen (○——○) of rats after subcutaneous msec for 10 sec, frequencies in shocks/sec. Time marks, 1 min. Uppermost record: effect of syrosingopine (4 mg/kg). Each point represents the mean from at least six rats. Noradrenaline content and sympathetic transmission are represented on the ordinate axis as percentages of control values. Values lying outside the range 80 to 120% are significantly different from the controls.

intravenously at 4 μg/min for 20 min from a Palmer constant-rate infusion pump. At 20 min after stopping the infusion the rats were killed and the hearts and spleens were removed and stored. The infusion of noradrenaline was made at such a time that each rat had been anaesthetized for 2 hr when killed; urethane anaesthesia itself produces little change in catechol amine content of the heart in this time (Spriggs, 1965).

Effects on adrenergic transmission. Noradrenaline was infused into anaesthetized rats at 1 μg/min for 20 min. Adrenergic transmission was assessed between 5 and 30 min after stopping the infusion.

Drugs

The doses of the following are expressed as base: bretylium tosylate, syrosingopine (dissolved in the solvent described by Pletscher, Shore & Brodie, 1955), physostigmine salicylate (−)-noradrenaline bitartrate and (−)-adrenaline. The doses of the following are expressed as salt: guanethidine hemisulphate, bethanidine hemisulphate and dexamphetamine sulphate.

RESULTS

The effect of guanethidine, bretylium, bethanidine or syrosingopine on the noradrenaline content of heart and spleen at various times after a single injection of drug is shown in Fig. 1. Both guanethidine (15 mg/kg) and syrosingopine (4 mg/kg) induced large

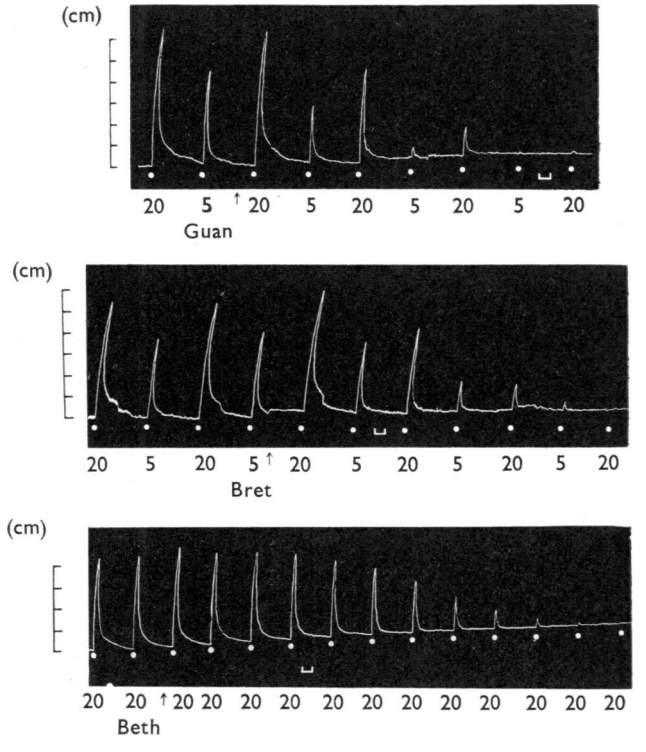

Fig. 2. Contractions of the inferior eyelid of anaesthetized rats to supramaximal preganglionic stimulation of the superior cervical sympathetic nerve. Parameters of stimulation; 5 V, 0.5 msec for 10 sec, frequencies in shocks/sec. Time marks, 1 min. Uppermost record: effect of guanethidine (Guan, 15 mg/kg) given subcutaneously at the arrow. Middle record: effect of bretylium (Bret, 15 mg/kg) given subcutaneously at the arrow. Lowest record: effect of bethanidine (Beth, 10 mg/kg) given subcutaneously at the arrow.

sustained depletions of noradrenaline similarly from both tissues, the depletions being more rapid in onset and of longer duration after syrosingopine. No change in tissue content occurred up to 18 hr after bretylium (15 mg/kg). A small (30%) loss of noradrenaline from both tissues was evident 4 hr after bethanidine (10 mg/kg) and lasted at least for a further 14 hr.

Sympathetic transmission

Guanethidine, bretylium and bethanidine each impaired sympathetic transmission, as measured by the response to physostigmine (Fig. 1). The onset of blockade was more rapid after guanethidine (90.6% inhibition after 15 min) than after bretylium or bethanidine (40% inhibition after 15 min). The duration of the blockade was longer after guanethidine than after bretylium or bethanidine (Fig. 1).

Within 1 hr of injecting syrosingopine a small decrease in the pressor response to physostigmine occurred but the response was not further impaired during the next 5 hr. In rats treated 24 hr beforehand with syrosingopine, the first response to physostigmine was not reduced, but succeeding responses were progressively smaller.

The responses of the inferior eyelid to sympathetic nerve stimulation were abolished 20 to 45 min after injecting guanethidine, bretylium or bethanidine (Fig. 2). A small reduction of the contractions of the eyelid often occurred during the first 2 hr after syrosingopine (Fig. 3,*a*). 24 hr after syrosingopine, however, contractions of the inferior eyelid were usually smaller than the contractions of eyelids of untreated rats (Fig. 3, *b* and *c*).

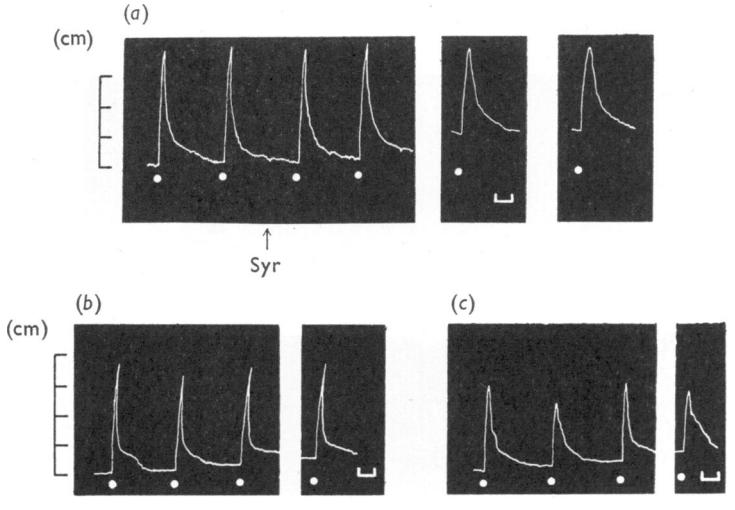

Fig. 3. Effect of syrosingopine (Syr) on contractions of the inferior eyelid of anaesthetized rats to supramaximal preganglionic stimulation of the superior cervical sympathetic nerve. Parameters of stimulation: 5 v, 0.5 msec, 20 shocks/sec for 10 sec. Time marks, 1 min. (*a*) Syrosingopine (4 mg/kg) given subcutaneously at the arrow; the second and third panels were recorded 60 and 120 min respectively after the injection. (*b*) and (*c*) Rats treated with syrosingopine 24 hr before the first panels; the second panels were recorded 30 min after the first ones.

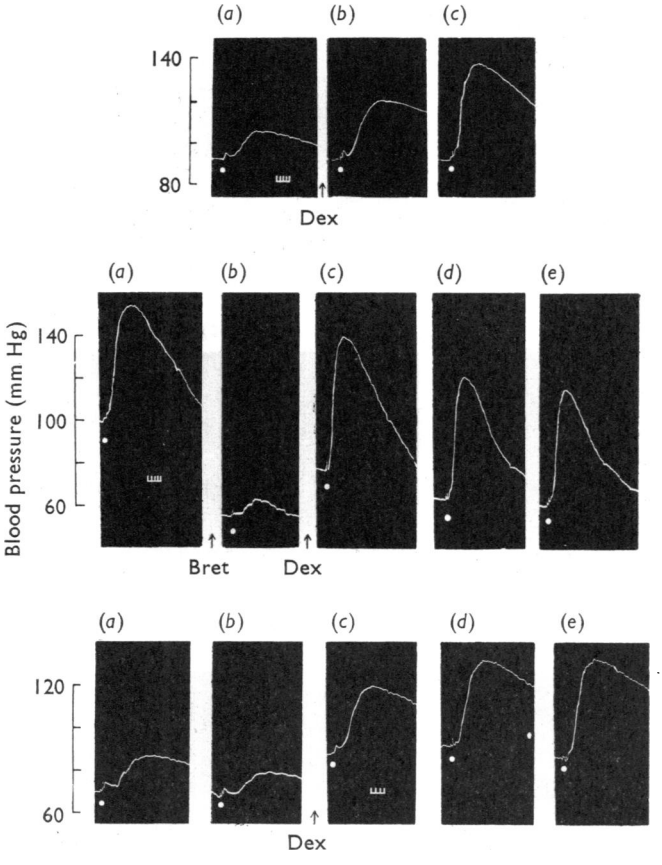

Fig. 4. Antagonism with dexamphetamine (Dex) of adrenergic-neurone blockade. Recordings of blood pressure from the carotid artery of rats anaesthetized with urethane. 20 μg of physostigmine were given intravenously every 30 min (at white dots). Time marks, minutes. Uppermost record: rat treated with guanethidine (15 mg/kg) 24 hr before (*a*); (*b*) and (*c*) were recorded 20 and 80 min respectively after the intravenous administration of dexamphetamine (Dex, 400 μg). Middle record: (*b*) was recorded 60 min after the subcutaneous injection of bretylium (Bret, 15 mg/kg); (c), (*d*) and (*e*) were recorded 20, 50 and 110 min respectively after the intravenous injection of dexamphetamine (100 μg). Lowest record: rat treated with bethanidine (10 mg/kg) 3 hr before (*a*); (*b*) was recorded 30 min after (*a*); (*c*), (*d*) and (*e*) were recorded 10, 70 and 130 min respectively after the intravenous administration of dexamphetamine (400 μg).

Antagonism of block

Dexamphetamine prevented the progressive reduction in the response to physostigmine in two rats given syrosingopine 24 hr beforehand but was ineffective in two other rats similarly treated with syrosingopine.

Dexamphetamine (100 to 400 μg, intravenously) antagonized the sympathetic blockade induced by guanethidine, bretylium or bethanidine, as shown by an increased pressor response to physostigmine and the recovery of eyelid contractions to sympathetic nerve stimulation (Figs. 4 and 5). In untreated rats dexamphetamine (400 μg, intravenously)

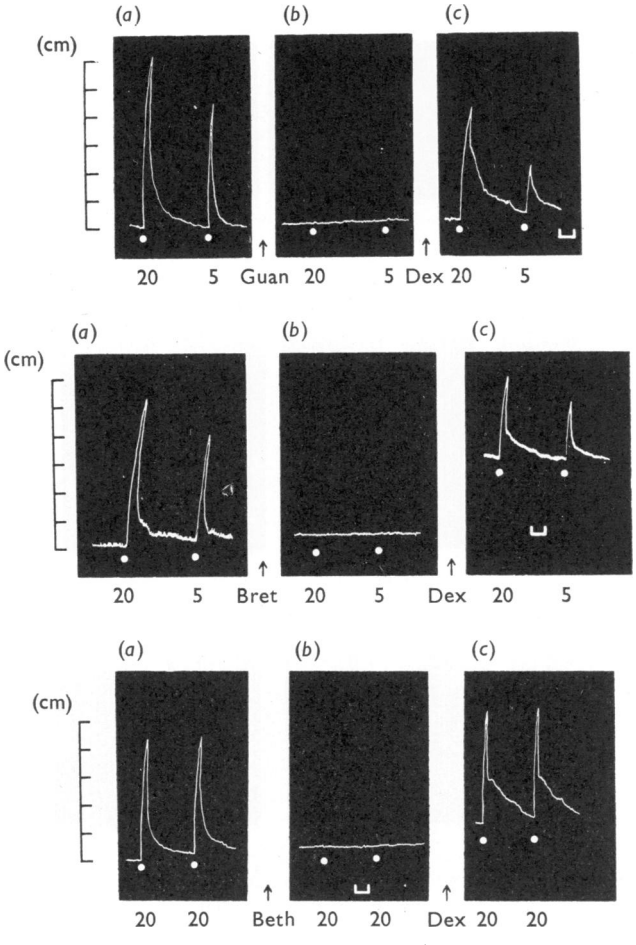

Fig. 5. Antagonism by dexamphetamine (Dex) of adrenergic-neurone blockade. Contractions of the inferior eyelid of anaesthetized rats to supramaximal preganglionic stimulation of the superior cervical sympathetic nerve. Parameters of stimulation: 5 V, 0.5 msec for 10 sec, frequencies in shocks/sec. Time marks, 1 min. Uppermost record: between (*a*) and (*b*) guanethidine (Guan, 15 mg/kg) was given subcutaneously; (*c*) was recorded 60 min after the intravenous administration of dexamphetamine (400 μg). Middle record: between (*a*) and (*b*) bretylium (Bret, 15 mg/kg) was given subcutaneously; (*c*) was recorded 60 min after the intravenous administration of dexamphetamine (100 μg). Lowest record: between (*a*) and (*b*) bethanidine (Beth, 10 mg/kg) was given subcutaneously; (*c*) was recorded 60 min after the intravenous administration of dexamphetamine (200 μg).

did not alter the pressor response to physostigmine nor the size of the contraction of the eyelid to sympathetic nerve stimulation.

In rats treated with guanethidine, bretylium, bethanidine or syrosingopine, noradrenaline infusions did not increase the response to physostigmine more than did a similar infusion in untreated rats.

Noradrenaline infusions and tissue noradrenaline levels

In untreated rats, an infusion of noradrenaline significantly increased the noradrenaline content of the heart. A larger increase occurred in rats given bretylium 60 min beforehand but a smaller increase occurred in rats given guanethidine 30 min beforehand (Fig. 6). In rats given bretylium 12 hr beforehand, guanethidine 24 hr beforehand, syrosingopine 1 hr beforehand or syrosingopine 48 hr beforehand, the increase in noradrenaline content of the heart after an infusion of noradrenaline was not different from that of the untreated control animals (Fig. 6). In the last three groups, however, the noradrenaline content of the heart before the infusion was significantly reduced, and the content after the infusion did not exceed the normal content of hearts from untreated control rats (Fig. 6).

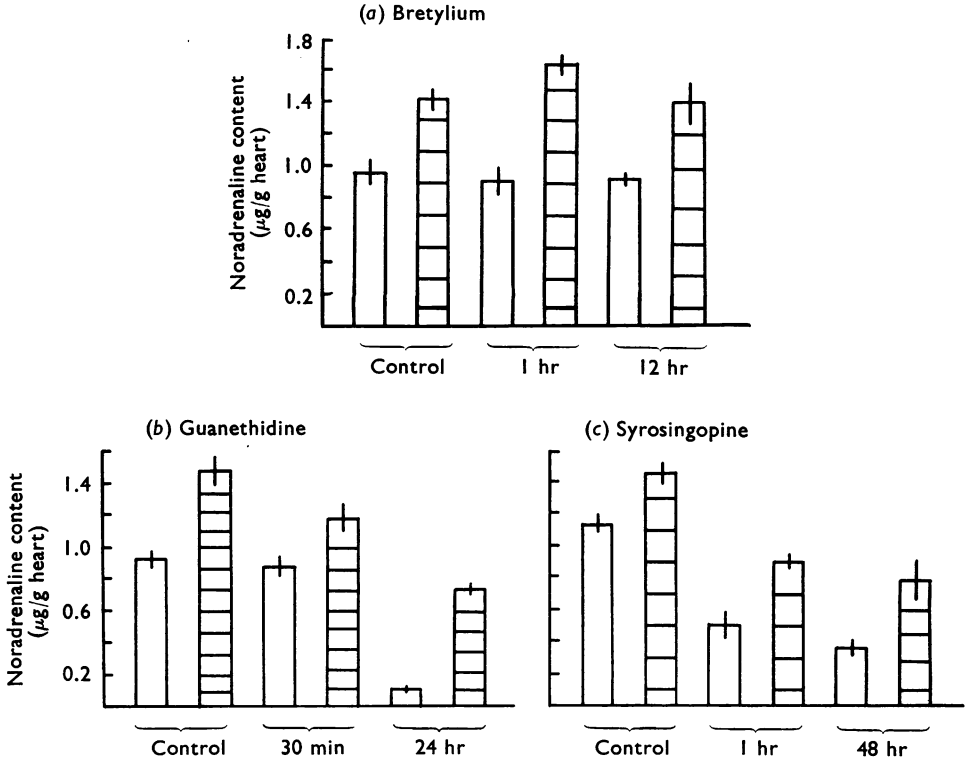

Fig. 6. The effect of (*a*) bretylium (15 mg/kg), (*b*) guanethidine (15 mg/kg) and (*c*) syrosingopine (4 mg/kg) on the uptake of noradrenaline by the heart in anaesthetized rats. Open columns: before infusion; hatched columns: after infusion. Each column represents the mean from six hearts and the standard errors are shown by the vertical lines. Times refer to the interval between the injection of drug and the commencement of the noradrenaline infusion.

The noradrenaline content of the spleen was also estimated, but the uptake of noradrenaline, even in untreated rats, was small and irregular. In contrast, the uptake of noradrenaline by the heart was significant ($P<0.02$) for each group.

DISCUSSION

The results show that the sympathetic blocking actions of guanethidine, bretylium and bethanidine are not related to a loss of noradrenaline from tissue stores. Thus 30 min after the administration of the blocking drug, full sympathetic blockade is established before a significant depletion of catechol amines has occurred. At this time noradrenaline infusions fail to restore sympathetic transmission even though noradrenaline is taken up by the heart.

After guanethidine the time course for the recovery of sympathetic function is similar to that for the repletion of heart noradrenaline but the restoration of sympathetic transmission is independent of the size of the noradrenaline stores. Thus 24 hr after guanethidine, when sympathetic transmission is approximately half of the control value, noradrenaline infusions replete the stores to the control level and yet fail to antagonize the adrenergic blockade. Although this latter finding appears contradictory to earlier claims (Cass & Spriggs, 1961), in untreated rats an infusion of noradrenaline results in a potentiation of the response to physostigmine similar to that observed by these workers in some guanethidine-treated rats. It is possible that by increasing the content of the noradrenaline stores more noradrenaline is released per impulse by active (that is, unblocked) adrenergic nerves. Thus the fewer fibres blocked by guanethidine the larger the potentiation by a noradrenaline infusion of the response to physostigmine.

Day & Rand (1963) have shown that dexamphetamine antagonizes an adrenergic blockade induced, shortly beforehand, by guanethidine. In the present experiments dexamphetamine rapidly and persistently antagonized the guanethidine-induced sympathetic block, even when the tissue noradrenaline stores were depleted. The sympathetic blockade induced by bretylium or bethanidine also was readily antagonized by dexamphetamine and not by noradrenaline infusions.

Bretylium, bethanidine and guanethidine have many properties in common (Boura & Green, 1962, 1963) and, as shown in this paper, the differences in their chemical structure do not greatly alter their adrenergic-neurone blocking activity but do significantly change their amine-depleting capacities. It appears therefore that amine-depleting activity is not an important factor in the adrenergic-neurone blockade induced by these drugs.

These findings prompted a reinvestigation of the significance of noradrenaline depletion in the sympathetic blockade induced by reserpine. An analogue of reserpine, syrosingopine, was selected as it lacks central depleting activity which might otherwise have altered the central mechanism of the pressor response to physostigmine. Syrosingopine is equiactive with reserpine in depleting peripheral stores of noradrenaline (Orlans, Finger & Brodie, 1960).

Syrosingopine depleted more than half of the noradrenaline from heart and spleen within 1 hr and induced full depletion within 4 hr. During the first hour, a small decrease in the response to physostigmine and in the contractions of the inferior eyelid to sympathetic nerve stimulation occurred, but there was no further decrease during the next 4 hr. Since the depleting activity of syrosingopine is not accompanied by a significant adrenergic blockade it appears that loss of noradrenaline from tissue stores *per se* does not induce failure of adrenergic transmission. At 24 hr after syrosingopine, a progressive decrease in successive responses to physostigmine occurred ; this was not prevented by noradrenaline infusions, even though these repleted the tissue stores, but

was stemmed, in two out of four experiments, by dexamphetamine. Day (1962) in one experiment observed that contractions of the cat nictitating membrane to sympathetic nerve stimulation, 24 hr after reserpine (5 mg/kg), were very small but that they were increased after dexamphetamine (1 mg/kg) and not by an infusion of noradrenaline. It is possible that the adrenergic-neurone blocking action of syrosingopine is similar to that of guanethidine, bethanidine and bretylium, and as such is independent of depletion of noradrenaline stores.

In 1958, Muscholl & Vogt found that adrenergic transmission in rabbits was not impaired when 75% of the noradrenaline content of the superior cervical ganglion had been depleted by reserpine. In conjunction with the recent evidence of Andèn & Magnusson (1963) and Carlsson (1964) that (+)-adrenaline, metaraminol and α-methylmetatyrosine induce greater than 95% depletion of tissue noradrenaline without impairing sympathetic transmission, it appears that sympathetic transmission may continue in the absence of stores of noradrenaline and that synthesis of noradrenaline within the nerve terminals keeps pace with immediate demands. There is considerable evidence that noradrenaline synthesis is rapid. In the brain, noradrenaline is doubled within 4 hr of giving a monoamine oxidase inhibitor (Brodie & Costa, 1962) indicating that the whole store of noradrenaline is normally turned over within this time period. Spriggs (1965) has shown that noradrenaline depleted from heart and adrenal glands during anaesthesia is rapidly restored as anaesthesia proceeds, illustrating that a rapid turnover of catechol amines occurs in peripheral tissues.

Many workers have found that the uptake of noradrenaline into tissue stores is inhibited in the presence of guanethidine, bretylium or reserpine (Hertting, Axelrod & Whitby, 1961 ; Hertting, Axelrod & Patrick, 1962 ; Iversen, 1965), but they used small concentrations of noradrenaline. In the present experiments, the amounts of noradrenaline infused were relatively large, though comparable with the amounts used by Burn & Rand (1958, 1960a, b). It appears that two uptake mechanisms may be involved. One which is saturated by small concentrations of noradrenaline (Dengler, Spiegal & Titus, 1961) and which is impaired by many drugs (Iversen, 1964, 1965) and the other which is able to handle much larger concentrations of noradrenaline. The present results show that the second uptake mechanism is not readily impaired by guanethidine, bretylium or syrosingopine. It is unlikely that noradrenaline passively enters the cardiac stores by diffusion as uptake is not observed in the spleens of these animals.

The possibility exists that the mechanism concerned with taking up these large concentrations of noradrenaline passes the noradrenaline into stores which are different from those in which noradrenaline is usually stored and from which it is released by nerve impulses. This is unlikely, however, as Burn and Rand (1960b) have shown that responses to sympathetic stimulation in untreated animals are potentiated by noradrenaline infusions and in the present experiments, using untreated rats, the response to physostigmine elicited after a noradrenaline infusion is larger than that preceding the infusion.

SUMMARY

1. The time courses of the effects of a single dose of bretylium, bethanidine, guanethidine or syrosingopine on adrenergic transmission and on the noradrenaline contents of heart and spleen of rats have been estimated and compared.

2. Dexamphetamine antagonized adrenergic blockade induced by each of the four drugs, irrespective of the size of the tissue noradrenaline store prevailing at the time.

3. Noradrenaline infusions failed to antagonize the adrenergic blockade induced by these four drugs even though the tissue noradrenaline stores were repleted by the infusions.

4. The importance of the noradrenaline store to the maintenance of adrenergic transmission in the rat is discussed.

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