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URINARY EXCRETION OF CATECHOL AMINES IN THE RAT AFTER THEIR LIBERATION BY RESERPINE OR DEXAMPHETAMINE

BY

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Daily urinary excretion of catechol amines in normal rats and in rats from which the adrenal medullae had been removed has been determined by a photofluorimetric method. In both groups, reserpine (2 mg/kg, intraperitoneally) produces: (1) A decrease in the urinary excretion of noradrenaline which persists for more than 3 weeks; this action is not influenced by monoamine oxidase inhibitors and mecamylamine. (2) An increase, within 20 to 68 hr, in the urinary excretion of adrenaline, even though the urine of rats without adrenal medullae does not usually contain adrenaline. These effects are prevented by monoamine oxidase inhibitors and, in the normal animals, are reduced by mecamylamine. In both groups, dexamphetamine (6 mg/kg, intraperitoneally) produces an increase in the excretion of adrenaline and noradrenaline, the adrenaline appearing in the urine of the rats without adrenal medullae within 20 to 44 hr. Mecamylamine prevents the effect of dexamphetamine on the excretion of noradrenaline. Dexamphetamine, administered within a week of reserpine treatment, produces its usual effects on the urinary excretion of catechol amines in normal rats, but has no effect in rats without adrenal medullae. The results are discussed with regard to both the mechanism by which reserpine and dexamphetamine influence the peripheral stores of adrenaline and noradrenaline, and the significance of the adrenal and extra-adrenal chromaffin system.

Variations in the functional activity of the sympathetic nervous system, whether physiological or physiopathological, are reflected, both in man and in the experimental animal, by similar modifications in urinary catechol amine output (Euler, 1956). Furthermore, in rats sympathetic hyperactivity produced by exposure to low temperature causes a slight reduction in the peripheral stores of the sympathetic mediators and a simultaneous increase in urinary catechol amines (Leduc, 1961). Treatment with reserpine depletes the peripheral stores of noradrenaline and produces functional inactivation of the sympathetic nervous system (Carlsson, Rosengren, Bertler & Nilsson, 1957). This is accompanied by a marked reduction in the urinary elimination of noradrenaline both in man and in experimental animals (Gaddum, Krivoy & Laverty, 1958; Kuschke & Ditfurth, 1958; Carlsson, Boje Rasmussen & Kristiansen, 1959; Bickel, Carpi & Bovet, 1961; Leduc, 1961).

In the present experiments the effects of reserpine and dexamphetamine on the urinary excretion of catechol amines have been studied, before and after administration of an amine oxidase inhibitor and a ganglion-blocking agent.

METHODS

Albino Wistar rats of either sex, weighing between 250 and 500 g, had food available for 4 hr and were then transferred for the next 20 hr to metabolism cages where only water was available *ad libitum*. Groups of six rats (two per cage) were trained in this way for 7 to 10 days, after which the urine from each cage was collected in bottles containing 6 ml. of 1 N-hydrochloric acid. The pooled samples from each group were analysed for their content of adrenaline and noradrenaline. Each single experiment represents values obtained from a group of six rats.

The urinary catechol amines were adsorbed on to an aluminium oxide column and eluted using the technique of Euler & Lishajko (1959); the eluates were then estimated fluorimetrically by the differential oxidation method of De Schaepdryver (1958). The recovery of urinary catechol amines was checked periodically by adding known amounts of adrenaline and noradrenaline to the urine; it was found to be 75 to 85%. Excretion figures have not been corrected for this loss.

In some rats during light ether anaesthesia, the adrenal medullae were removed from both adrenal glands through a lumbar incision. The adrenal cortex was incised and the medulla was removed by slight compression of the gland. The animals were treated with penicillin (10,000 U/day) for 4 days, and the experiments were started 7 to 45 days later. At the end of the experiments, complete absence of medullary tissue was confirmed by histological examination.

The following drugs were used: reserpine (Raudixoid), dexamphetamine, the monoamine oxidase inhibitor 1-(1,4-benzodioxan-2-ylmethyl)-1-benzylhydrazine (2596 I.S.) and mecamylamine. The drugs were injected intraperitoneally.

RESULTS

Effect of reserpine in normal rats. Table 1 and Fig. 1, *a* show the effects of reserpine (2 mg/kg) on the urinary excretion of catechol amine in normal rats. In confirmation of previous observations (Proosdij-Hartzema, 1959; Hazard, Beauvallet, Fugazza & Solier, 1960; Bickel *et al.*, 1961; Leduc, 1961) the excretion of adrenaline increased considerably during the 20 hr after injection of reserpine, then returned to normal levels in the subsequent 44 to 68hr. The elimination of noradrenaline was reduced, reaching a minimum 2 to 6 days after administration of reserpine; it remained low for as long as 3 weeks afterwards.

TABLE 1

EFFECTS OF RESERPINE (2 MG/KG), OF MECAMYLAMINE (20 MG/KG EVERY 6 HR, TOTAL DOSE 80 MG/KG) AND OF COMBINED DRUG TREATMENT ON THE URINARY EXCRETION OF CATECHOL AMINES IN THE RAT

Values are means and standard errors. * These values differ significantly from the controls ($P<0.01$). Significance of the difference of II versus III. Adrenaline: $P<0.01$; noradrenaline: $P>0.05$

	Treatment	No. of expts.	Urinary output (μg/kg/20 hr) of Adrenaline	Noradrenaline
I	Control	12	0·48±0·024	1·47±0·044
II	Reserpine 0–20 hr	4	1·06±0·062*	1·05±0·115*
III	Reserpine and mecamylamine 0–20 hr	3	0·68±0·044*	0·82±0·113*
IV	Mecamylamine 0–20 hr	5	0·45±0·035	1·43±0·074

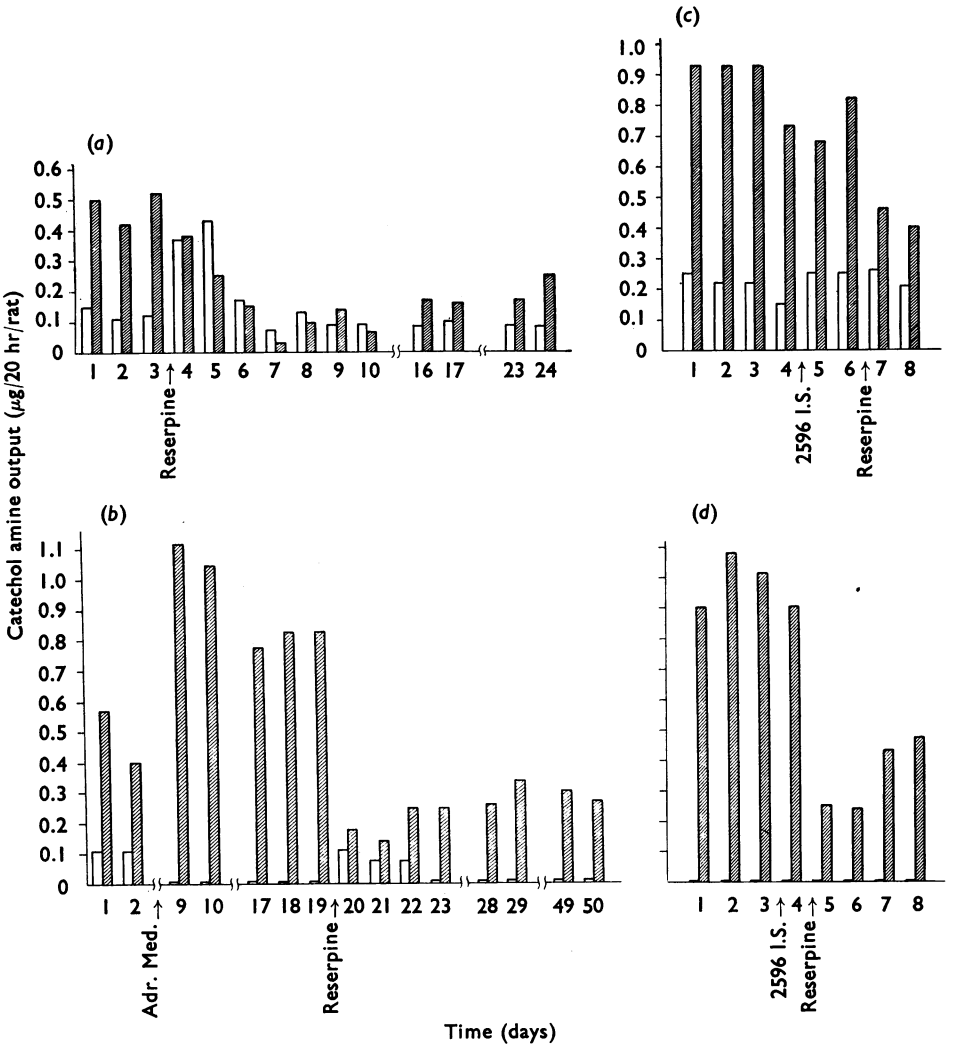

Fig. 1. Action of reserpine (2 mg/kg) on the urinary excretion of catechol amines, and the effect of 2596 I.S. on this action. (*a*) In the normal rat there is an increase in adrenaline output (open columns) and a persisting depression of noradrenaline excretion (shaded columns); six rats, average weight 320 g. (*b*) After removal of the adrenal medullae (at Adr. Med.), reserpine causes a reappearance of adrenaline excretion and a depression of noradrenaline output; six rats, average weight 470 g. (*c*) Prevention by a monoamine oxidase inhibitor (2596 I.S., 10 mg/kg) of the increase in adrenaline excretion provoked by reserpine in six normal rats, average weight 480 g. (*d*) As (*c*), but for six rats, average weight 350 g, with adrenal medullae removed, 10 days before the experiment.

Effect of removal of adrenal medullae on the action of reserpine. In a series of six experiments (Table 2), removal of the adrenal medullae decreased the urinary excretion of adrenaline to below measurable limits (less than 0.10 to 0.15 μg/kg/20 hr). Higher than normal levels of noradrenaline were excreted in three experi-

ments made during the 2 weeks following surgery, but normal levels of noradrenaline excretion were observed in three other experiments made 6 weeks after removal of the medullae (Table 2). When reserpine (2 mg/kg) was given to these animals, adrenaline appeared in the urine during the 20 to 68 hr after the injection. At the same time, there was a fall in urinary noradrenaline which lasted for 3 weeks. Similar effects were obtained in rats given reserpine 16 days (Fig. 1, *b*) or 45 days (Fig. 2, *e*) after demedullation.

TABLE 2

CATECHOL AMINE EXCRETION IN NORMAL RATS AND IN THOSE WITH ADRENAL MEDULLAE REMOVED

Values are means and standard errors. * This value differs significantly from the normal ($P<0.01$). (+)=Lower than the measurable limits (0·10 to 0·15 μg/kg/20 hr) of the method used

Treatment	No. of expts.	Urinary output (μg/kg/20 hr) of Adrenaline	Noradrenaline
Normal	32	0·49±0·023	1·45±0·041
5–10 days after removing medullae	3	(+)	2·63±0·437*
45–50 days after removing medullae	3	(+)	1·61±0·028

Action of a monoamine oxidase inhibitor. In confirmation of previous observations obtained with monoamine oxidase inhibitors (Bickel *et al.*, 1961), the treatment of animals with 2596 I.S. (10 mg/kg, intraperitoneally) prevented the effects of reserpine (given 24 to 48 hr later) on the urinary elimination of adrenaline, but did not alter the decrease in the excretion of noradrenaline induced by reserpine in normal rats (Fig. 1, *c*). Similar effects were obtained in rats without adrenal medullae (Fig. 1, *d*).

Action of a ganglion blocking agent. On the day of treatment, mecamylamine (20 mg/kg, every 6 hr, total dose 80 mg/kg) had no effect on the urinary excretion of catechol amines (Fig. 2, *a*, and 4, *a*; Table 1). In the succeeding days, however, there was an increase in the urinary elimination of adrenaline without much variation in that of noradrenaline. The rats which received both reserpine and mecamylamine (Fig. 2, *c*), excreted more adrenaline in the first 20 hr than did those rats treated only with mecamylamine. This increase, however, was less than that observed after the administration of reserpine alone (Fig. 2, *b*). In the succeeding days a more transient increase in the adrenaline excretion was observed in the animals treated with reserpine and mecamylamine than in those given mecamylamine alone. Treatment with mecamylamine did not affect the reduced output of noradrenaline in the urine induced by reserpine. The inhibitory action of mecamylamine on increased adrenaline excretion caused by reserpine was confirmed in two other experiments (Table 1).

Similar experiments were conducted on rats whose adrenal medullae had been removed 6 weeks previously and which were excreting normal amounts of noradrenaline (Fig. 2). Treatment with mecamylamine had no significant effect, as in the intact animals, on the elimination of urinary noradrenaline, nor did it alter

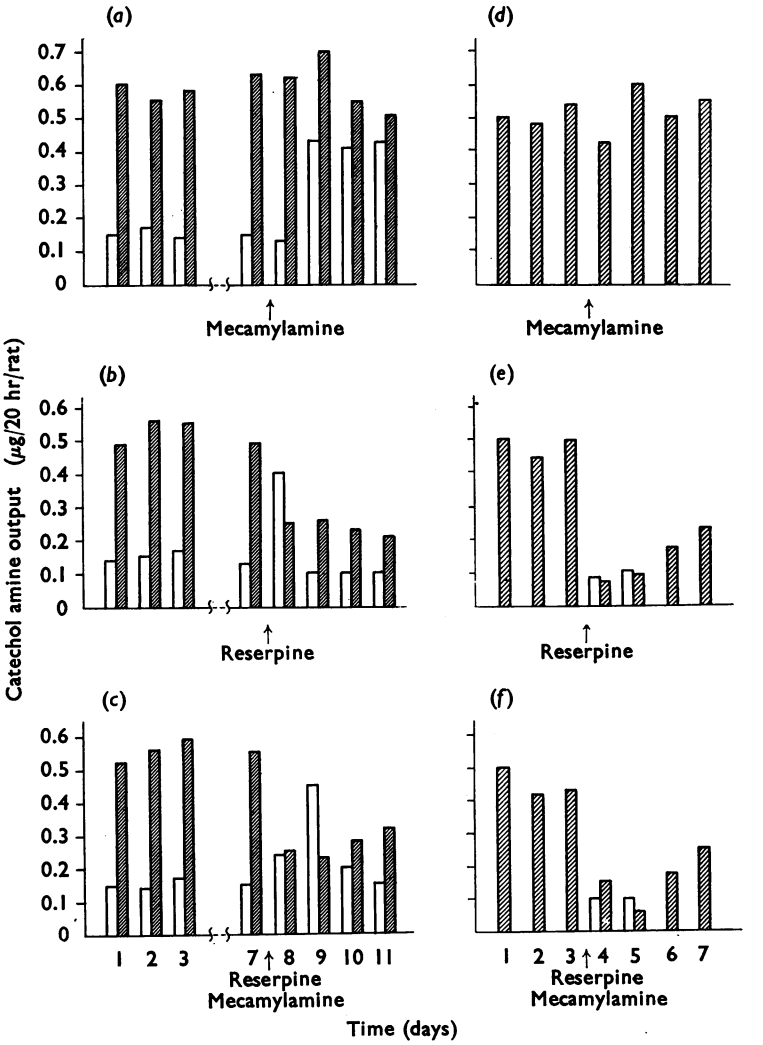

Fig. 2. (*a*), (*b*) and (*c*). Inhibition by mecamylamine (20 mg/kg every 6 hr, total dose 80 mg/kg) of the effects of reserpine on adrenaline excretion in normal rats. (*a*) Six rats, average weight 380 g; (*b*) six rats, average weight 350 g; and (*c*) six rats, average weight 380 g. (*d*), (*e*) and (*f*). Persistence of reserpine action on catechol amine excretion in rats with adrenal medullae removed (six weeks before the experiment) and treated with mecamylamine. (*d*) Six rats, average weight 320 g; (*e*) six rats, average weight 300 g; and (*f*) six rats, average weight 310 g. Symbols as in Fig. 1.

the effects of reserpine in these rats. Thus reserpine produced the reappearance of adrenaline within 48 hr and a prolonged reduction in the urinary excretion of noradrenaline.

Action of dexamphetamine in normal rats and in those with adrenal medullae removed. Dexamphetamine (3 mg/kg) given to normal rats produced a slight

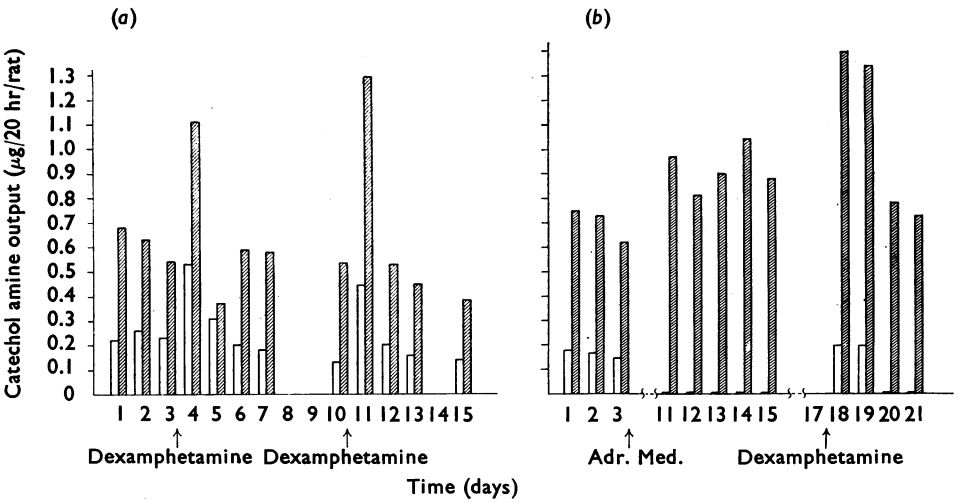

Fig. 3. Action of dexamphetamine (6 mg/kg) on catechol amine excretion. (*a*) In normal rats there is an increase of adrenaline and noradrenaline elimination; six rats, average weight 420 g. (*b*) In rats without adrenal medullae (Adr. Med) an increase in noradrenaline output and a reappearance of adrenaline excretion is observed; six rats, average weight 290 g. Symbols as in Fig. 1.

increase in the urinary elimination of catechol amines, whereas with 6 mg/kg (Fig. 3, *a*) the increase was larger. The effects of dexamphetamine (6 mg/kg) were examined in a series of eleven experiments (Table 3); in ten of these, an increase was observed within 20 hr in the urinary excretion of both adrenaline and noradrenaline; in one experiment, only the adrenaline excretion was increased.

In rats without adrenal medullae given dexamphetamine (6 mg/kg) there was an increase in noradrenaline elimination, and adrenaline appeared in the urine within 20 to 44 hr (Fig. 3, *b*).

Action of a ganglion-blocking agent. During the first day, administration of mecamylamine (20 mg/kg every 6 hr, total dose 80 mg/kg) and dexamphetamine (6 mg/kg) simultaneously with the first injection of mecamylamine, produced an

TABLE 3

EFFECTS OF DEXAMPHETAMINE (6 MG/KG I.P.) AND OF DEXAMPHETAMINE COMBINED WITH MECAMYLAMINE (20 MG/KG I.P. EVERY 6 HR, TOTAL DOSE 80 MG/KG) ON THE URINARY EXCRETION OF CATECHOL AMINES IN THE RAT

Values are means and standard errors. * These mean values vary significantly from the control mean ($P<0.01$). Significance of the difference of II versus III. Adrenaline: $P>0.05$; noradrenaline: $P<0.05$

	Treatment	No. of expts.	Urinary output (μg/kg/20 hr) of Adrenaline	Noradrenaline
I	Control	15	0·49±0·039	1·52±0·080
II	Dexamphetamine 0–20 hr	11	1·23±0·143*	2·94±0·346*
III	Dexamphetamine and mecamylamine 0–20 hr	4	0·99±0·175*	1·57±0·069

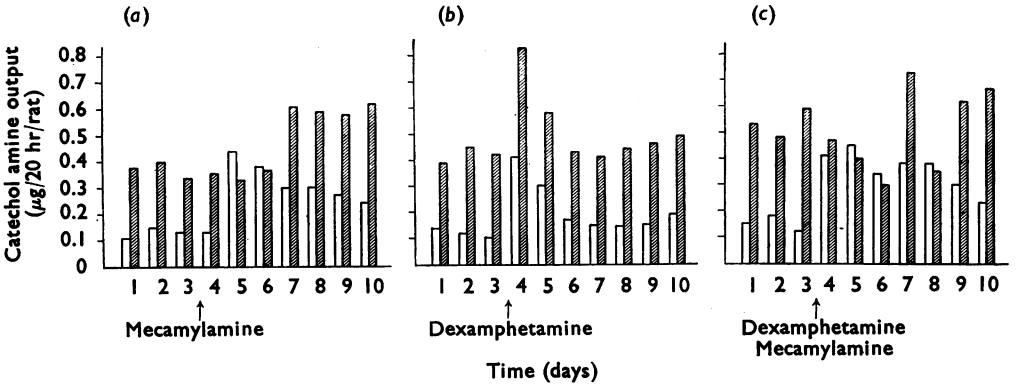

Fig. 4. Inhibition by mecamylamine (20 mg/kg every 6 hr, total dose 80 mg/kg) of the effect of dexamphetamine on noradrenaline excretion. (*a*) Six rats, average weight 290 g; (*b*) six rats, average weight 285 g; and (*c*) six rats, average weight 300 g. Symbols as in Fig. 1.

increased urinary elimination of adrenaline only without altering the levels of noradrenaline (Fig. 4). In the succeeding days, the urinary excretion of catechol amines was similar in animals given only mecamylamine or given mecamylamine and dexamphetamine. Similar results were obtained in three other experiments (Table 3).

Effect of reserpine. In normal rats, treatment with reserpine (2 mg/kg, 7 to 10 days beforehand) did not alter the dexamphetamine effect (Fig. 5, *a*). However, in rats without adrenal medullae, dexamphetamine administered 7 to 10 days after reserpine had no significant effect on noradrenaline excretion and caused the appearance of adrenaline in only two of three experiments (Fig. 5, *b*; Table 4). A second administration of dexamphetamine given 14 to 19 days after the reserpine caused a slight but significant increase in the elimination of noradrenaline but no change in adrenaline excretion (Table 4).

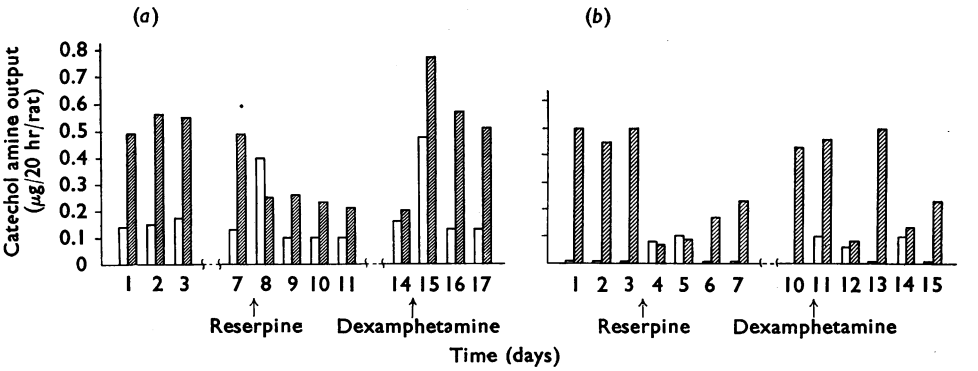

Fig. 5. (*a*) Persistence of the effects of dexamphetamine (6 mg/kg) on catechol amine excretion in normal rats treated with reserpine; six rats, average weight 350 g. (*b*) In rats with adrenal medullae removed 6 weeks before the experiment, treatment with reserpine prevents the effects of dexamphetamine on noradrenaline excretion; six rats, average weight 300 g. Symbols as in Fig. 1.

TABLE 4

EFFECTS OF DEXAMPHETAMINE (6 MG/KG) ON THE URINARY EXCRETION OF CATECHOL AMINES IN RATS WITHOUT ADRENAL MEDULLAE AND TREATED WITH RESERPINE (2 MG/KG)

The values (means and standard errors) indicate the differences between the excretion of the 20 hr after and that of the 20 hr before the administration of dexamphetamine. * This change is significant ($P<0.05$)

Time of dexamphetamine administration	No. of expts.	Changes in output (μg/kg/20 hr) due to dexamphetamine of	
		Adrenaline	Noradrenaline
7–10 days after reserpine	3	$+0.29\pm0.159$	-0.25 ± 0.217
14–19 days after reserpine	3	-0.04 ± 0.043	$+0.64\pm0.127$*

DISCUSSION

Action of reserpine and dexamphetamine on the urinary catechol amines

Our results with reserpine agree with those obtained in more specialized studies concerning the central and the peripheral actions of this drug. Thus the fall in noradrenaline excretion following administration of reserpine reflects the exhaustion of the peripheral stores of the sympathetic mediator (Gaddum *et al.*, 1958) and the resulting functional inactivation of the sympathetic pathways with noradrenaline as transmitter. Reserpine lowers the noradrenaline content of peripheral tissues primarily by a direct action (Shore, 1962); this effect is present also in animals treated with ganglion-blocking agents (Hertting, Potter & Axelrod, 1962) or with monoamine oxidase inhibitors (Carlsson *et al.*, 1957). In accordance with these results, we have found that reserpine reduces the noradrenaline excretion even in rats treated with mecamylamine or 2596 I.S.

The adrenaline content of the adrenal gland is only moderately depleted by the doses of reserpine here employed (Callingham & Mann, 1962); therefore there is no functional inactivation of the adrenal medulla. Indeed, reserpine causes an increased adrenaline excretion which is reduced by the administration of mecamylamine. Therefore, this increased urinary excretion of adrenaline must be caused by a central action of reserpine leading to an increased sympathetic nervous activity and, consequently, to a functional liberation of adrenaline, the only sympathetic mediator available under these conditions. Other evidence has been given for this central activation of the sympathetic system. Thus hexamethonium reduces the increased urinary elimination of total catechol amines in rats chronically treated with reserpine (Mirkin, 1961); moreover an increased preganglionic sympathetic activity has been observed in cats treated with reserpine (Iggo & Vogt, 1960). This activation of the sympathetic system may in some way be connected with the central depressant action of reserpine. Thus 2596 I.S. prevents the central depressant effects of reserpine (Bovet-Nitti, Orsingher, Landi-Vittory & Bovet, 1961) and also prevents the increase in urinary excretion of adrenaline.

The increased elimination of catechol amines caused by dexamphetamine may be referred either to the peripheral liberating action characteristic of the non-

phenolic sympathomimetic amines (Burn & Rand, 1958) or to the central stimulation typical of dexamphetamine. We assume that the first mechanism can be excluded because (1) this type of liberation appears to occur, in the normal animal, at the sympathetic nerve endings exclusively (Vane, 1960 ; Stjärne, 1961 ; Weiner, Draskóczy & Burack, 1962) and therefore the increased adrenaline elimination caused by dexamphetamine cannot be explained by this mechanism ; (2) neither tyramine nor phenylethylamine are capable of exercising similar effects on the urinary catechol amines except in almost toxic doses (50 to 100 mg/kg ; Biscardi & Carpi, unpublished) ; and (3) the effects of mecamylamine also seem to indicate a central origin of these changes. The failure of mecamylamine to inhibit increases in urinary excretion of adrenaline is possibly because the chromaffin cells of the adrenal medulla are more resistant to the action of ganglion-blocking agents than the synapses of the sympathetic nervous system (Spoerel & Gowdey, 1956 ; Leduc, 1961). Consequently, while mecamylamine can limit the effects of functional stimulation on the adrenal medulla such as is produced by reserpine, it does not influence the stronger stimulation of central origin caused by dexamphetamine.

The less intensive blocking action of mecamylamine on the chromaffin cells could also explain the delayed increase in adrenaline excretion provoked by this drug. In agreement with Leduc (1961), who observed similar effects, there is, following the ganglion block, a compensatory activation of the preganglionic sympathetic system ; since the adrenal medulla is more resistant to ganglion-blocking agents this reaction is more effective on the chromaffin cells than on the ganglion synapses.

The functional significance of the adrenal and extra-adrenal chromaffin system

The increased noradrenaline elimination during the 2 weeks following removal of the adrenal medullae demonstrates a transitory sympathetic hyperactivity possibly linked with the surgical stress, aggravated by the sudden deficit of medullary hormones. The subsequent recovery of noradrenaline excretion to normal levels and the persistent absence of adrenaline elimination agree with the observations of Crawford & Law (1958).

The functional significance of the extramedullary chromaffin tissue is uncertain (Muscholl & Vogt, 1964). A lack of reactivity of this tissue to drugs acting through the autonomic nervous system is suggested by the experiments of Crawford & Law (1958) on rats without adrenal medullae and of De Schaepdryver, Preziosi & Van Der Stricht (1959) on similar dogs. However, other experiments indicate that the extra adrenal chromaffin tissue may respond to stimuli. Thus nicotinic drugs cause catechol amine release from perfused paraganglia in the puppy (Muscholl & Vogt, 1964) ; exposure to cold is followed by increased adrenaline elimination in adrenalectomized rats (Leduc, 1961). Other evidence for this functional reactivity of the extramedullary chromaffin system is given by the observation that measurable quantities of adrenaline appear in the urine of rats without adrenal medullae and treated with reserpine or dexamphetamine. The lack of influence of mecamylamine on the effects of reserpine in rats without medullae should be considered with certain reservation because, even in the normal rat, the inhibiting action of mecamylamine on the increased urinary excretion of adrenaline produced by reserpine

appears to be incomplete. Further work will have to establish whether these discrepant conclusions are because different pharmacological agents and different species were used.

Finally, the marked difference in the action of dexamphetamine in normal rats and in animals with adrenal medullae removed given reserpine 7 days previously, deserves particular consideration. In the latter animals, the absence of the usual increase in urinary noradrenaline after injection of dexamphetamine suggests that the stores of this mediator are so greatly impoverished by reserpine that they cannot sustain the prolonged phase of sympathetic hyperactivity which causes an increase in the urinary elimination of noradrenaline. Since the increase in urinary noradrenaline produced by dexamphetamine in normal rats is still evident after a week of treatment with reserpine, it has to be assumed that, in rats without their adrenal medullae, the capacity for synthesis of the sympathetic mediator is greatly reduced compared with that in normal rats. This indicates, in agreement with the observations of Burn & Rand (1960), that the adrenal chromaffin system not only holds rich deposits of sympathetic mediators at the disposition of the organism in cases of emergency, but it is also the organ which helps in the supply of mediators to the entire sympathetic system.

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