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CHANGE OF VASOMOTOR RESPONSE TO CATECHOL AMINES ON REPEATED ADMINISTRATION

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Catechol amines injected intravascularly affect many organs, and especially the cardiovascular system. Since some of these actions, such as the rise in blood pressure, are of therapeutic importance, it seemed of interest to study the constancy of the vasomotor response on repeated administration. On the one hand, Rosenthale & DiPalma (1962), Pohle & Matthies (1963) and Pérez-Reyes & Lipton (1963) have reported the development of tachyphylaxis when large doses of noradrenaline or adrenaline were repeatedly injected. On the other hand, the response to stimulation of adrenergic nerves has been shown to increase after infusion of noradrenaline (Burn & Rand, 1960; Mirkin & Euler, 1963), a potentiation ascribed to loading of nerve endings with this catechol amine. It seems that tissue stores are not saturated under normal conditions, since considerable increases in content may follow administration of various catechol amines (Siegel, Gilmore & Sarnoff, 1961; Strömblad & Nickerson, 1961; Harrison, Levitt & Udenfriend, 1963; Muscholl & Maître, 1963). It has been suggested that tissue uptake and storage are important factors in the removal of circulating catechol amines (Axelrod, Weil-Malherbe & Tomchick, 1959; Burn & Rand, 1960; Whitby, Axelrod & Weil-Malherbe, 1961; Kirpekar, Cervoni & Furchgott, 1962). Blockade of tissue uptake may, therefore, be the mechanism of catechol amine potentiation by cocaine, because a larger proportion of the injected dose can reach the receptors (Strömblad, 1960; Muscholl, 1961). A similar reasoning may be applied to the increased sensitivity toward catechol amines following denervation (Strömblad, 1960; Kirpekar *et al.*, 1962), when storage capacity in tissues is extremely low (Hertting, Axelrod, Kopin & Whitby, 1961). It has also been demonstrated that the capacity of catechol amine uptake from the circulation increases when the tissue concentration is low (Siegel *et al.*, 1961; Gillis, 1963).

These considerations have led us to test the response to injections of various catechol amines when administered before and after loading with the same or other catechol amines.

METHODS

One hundred and seventeen cats of either sex, weighing 2 to 4.5 kg, were used. Most operative procedures were carried out during ether anaesthesia; in a few instances, pentobarbitone sodium (35 mg/kg, intramuscularly) was used. Cannulae were introduced into the trachea, a femoral vein and a carotid artery. In all animals, bilateral vagotomy was performed, followed, in most, by spinal transection at C1. In some cats, bilateral adrenalectomy, preceding spinal transection, was carried out through a lateral approach.

The adrenal vessels were carefully tied before removal of the glands, in order to minimize the release of hormones into the circulation.

The animals were ventilated by a pump at constant rate and volume throughout the experiment. Blood pressure was recorded on kymograph paper by means of a mercury manometer. Rectal temperature was maintained at 37.5±0.5° C by appropriate heating of the animal. Unless stated otherwise, at least 1 hr elapsed between the end of operation and the start of the experiment proper.

The sectioned spinal cord was stimulated through bipolar electrodes touching the transection plane, with rectangular pulses (12 V, 10 to 20 shocks/sec, 5 msec duration) from a Tektronix stimulator, monitored on a Tektronix oscilloscope. Stimulation was intermittent (1 min on, 1 min off) and extended over 5 min.

Drugs were continuously infused from a Palmer pump, usually into the femoral vein. In some experiments adrenaline or noradrenaline was infused into the aorta through a polyethylene tube, introduced through the left carotid or left brachial arteries.

The following drugs were used: (−)-adrenaline hydrochloride (Hillel, Haifa), (−)-noradrenaline bitartrate (Winthrop Products), (±)-isoprenaline sulphate (Teva, Jerusalem), dopamine hydrochloride (Mann Research Laboratories), cocaine hydrochloride (Nederlandsche Cocaine Fabriek), and succinylcholine (L. Light & Co.). Doses are expressed as the salts.

RESULTS

Adrenaline. The blood pressure response to adrenaline increased progressively upon repeated administration. This is evident from Fig. 1, where (*a*) shows the response in the first series of injections, while (*b*) and (*c*) illustrate the third and fourth series, 15 and 25 min

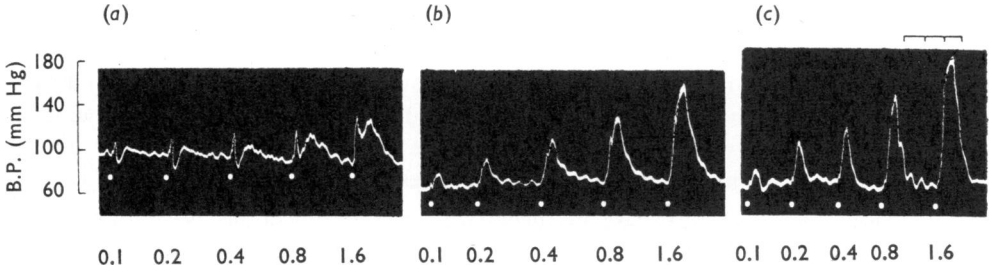

Fig. 1. Influence of repeated applications of adrenaline on its vasomotor effects in the spinal cat. (*a*), First; (*b*), third; and (*c*), fourth series of intravenous injections (second series not shown). White dots indicate times of injection of drug. Ordinate, blood pressure (B.P.). Time (in min) at upper right. Numbers denote doses in μg/kg.

respectively, after the end of the first series (see also Fig. 3,*a* and *c*). Furthermore, repetition of the lowest (first) dose immediately after completion of a series of injections regularly showed an increased pressor response relative to that observed on initial application of the same dose. After five to eight series of injections, no further augmentation of blood pressure response could be obtained. Qualitatively the same effect was observed when infusions of adrenaline (20 μg/kg) were given during the intervals between consecutive series of adrenaline injections. By this method, however, the maximal enhancement of the response was attained earlier.

It is also noteworthy that the depressor reactions, obtained with the lower doses of the drug in the first series, changed into blood pressure rises in the later series of injections.

Similarly, biphasic responses were converted in later series into pure pressor reactions (Fig. 1). The same phenomenon occurred also when the basal blood pressure was stable and did not fall as in Fig. 1. An analogous increase in response to adrenaline was also evident on comparing the level of blood pressure attained during successive 10-min periods of infusion.

Similar increments in the blood pressure rise and conversion of hypotensive into hypertensive reactions in response to adrenaline injection were observed after infusion of other catechol amines, such as noradrenaline, dopamine and isoprenaline (Fig. 2). In experiments

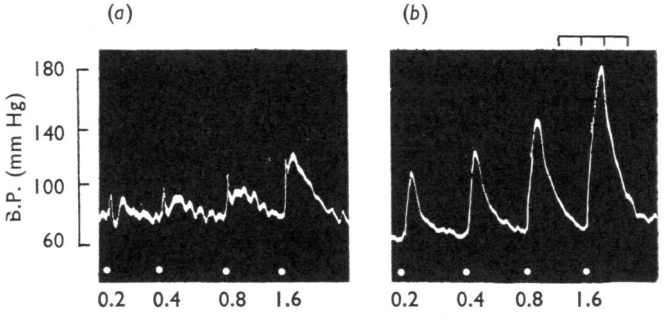

Fig. 2. Effect of isoprenaline infusion on blood pressure responses to adrenaline in the spinal cat. (*a*) and (*b*), Consecutive series of adrenaline injections. Between (*a*) and (*b*), isoprenaline, 20 μg/kg, was infused intravenously during 10 min. Notations as in Fig. 1.

of this type the enhanced response to adrenaline cannot be ascribed to repeated administration of adrenaline itself, because the augmentation of the response in two successive series of adrenaline injections was much smaller than the one found when catechol amines were infused during the interval between the two series.

The increased blood pressure rise in successive series of injections is summarized in Fig. 3 (▲ and ●). It should be noted here that the tachycardic action of adrenaline showed a different behaviour, being constant during repeated applications of the drug (Fig. 4).

Noradrenaline. The response to noradrenaline was studied in nine animals. The augmentation of the pressor responses was not confined to adrenaline. Thus, the level of blood pressure attained during successive infusions of noradrenaline was raised steadily (Fig. 5). Similarly, the response to single injections of noradrenaline was increased by infusion of adrenaline during the interval.

Isoprenaline. The effect of isoprenaline was studied in eight animals. The fall in blood pressure caused by a small dose of isoprenaline (0.2 μg/kg) was replaced by a rise in blood pressure after repeated administrations of this drug (Fig. 6).

Interval between series. A moderate, spontaneous increase of the hypertensive response to adrenaline was also observed when a long interval of time separated two series of injections. This was studied in seven animals. Fig. 3 demonstrates the increase in pressor reaction to adrenaline following an interval of 3 hr, compared to the response in the first series of injections. It should be noted that this interval is considerably longer than that necessary to achieve a maximal response when infusions of adrenaline are given. On

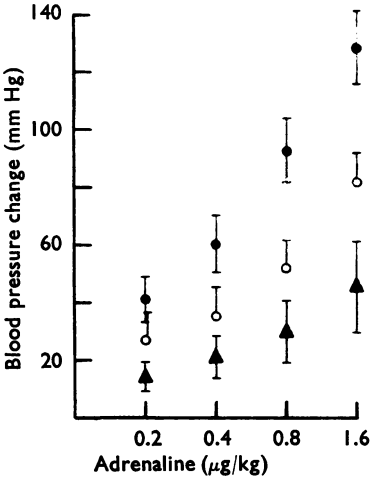

Fig. 3. Comparison of the increase in pressor response to adrenaline after a long interval without treatment, and that following repeated administration of adrenaline. Spinal cats, intravenous adrenaline injections. ▲, Responses in the first series of injections; ○, responses after an interval of 3 hr (no increase in response was observed by further extension of the time interval); and ●, maximal responses to injections of adrenaline after loading with this catechol amine (no further enhancement was observed by additional application of the drug). Vertical bars indicate standard deviations. Note that the potentiation following repeated administration of adrenaline (●) was achieved in a shorter time (1 hr) and exceeded considerably the increase obtained by a long rest (○).

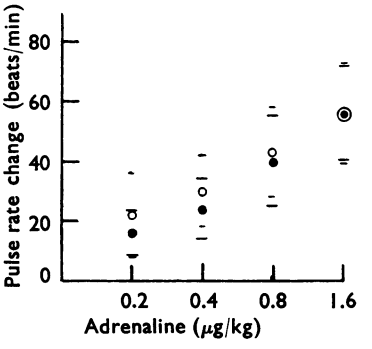

Fig. 4. Effect of adrenaline on heart rate. Spinal cats, adrenaline administered intravenously. ○, Responses to the first series of injections, with limits of standard deviation indicated by short horizontal bars; ●, responses after repeated application of adrenaline, when the maximal pressor effect had already been obtained (see Fig. 3,●), with limits of standard deviation indicated by long horizontal bars. Note that no significant difference is observed between the two series.

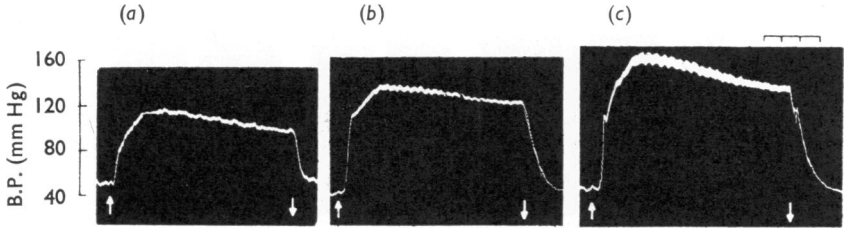

Fig. 5. Blood pressure response to repeated intravenous infusions of noradrenaline in the spinal cat. (*a*), Second; (*b*), fourth; and (*c*), fifth infusion. Noradrenaline, 3 μg/kg/min, was given in each infusion during 10 min. Arrows show start and end of infusions. Notation as in Fig. 1.

further lengthening of the interval (up to 6 hr) the response to adrenaline remained at a constant level. However, infusion of adrenaline at this stage could still augment considerably the reaction (Fig. 7). (In this particular case no significant change in response was observed during the long time interval (Fig. 7,*a* and *b*).) It is also evident from Fig. 3 that the response obtained after repeated injections of adrenaline (filled circles) exceeds that found after a long time interval (empty circles).

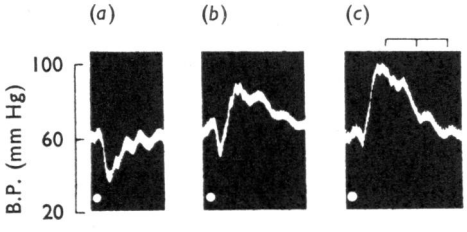

Fig. 6. Blood pressure response to isoprenaline on repeated administration in the spinal cat. (*a*), (*b*) and (*c*), 0.2 μg/kg of isoprenaline intravenously at white dots. Between (*a*) and (*b*), 30 μg/kg; between (*b*) and (*c*), 40 μg/kg of isoprenaline were infused intravenously. Notation as in Fig. 1.

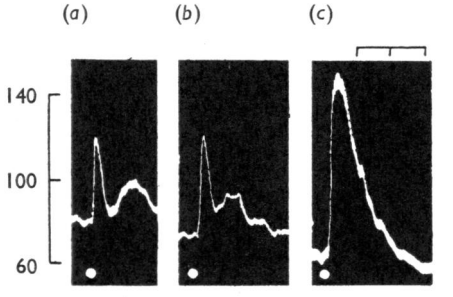

Fig. 7. Comparison of the effect of a long time interval and of adrenaline infusion on pressor reactions to intravenous adrenaline, 0.8 μg/kg at white dots. Between (*a*) and (*b*), an interval of 3 hr; between (*b*) and (*c*), infusion of adrenaline, 3 μg/kg/min for 10 min. Notation as in Fig. 1.

Adrenalectomy. The increased response after a long time interval might be due to supply of catechol amines from endogenous sources. It has been recently reported that the adrenals may provide catechol amines for stores in the heart and other tissues (Bhagat, 1963; Coleman & Glaviano, 1963; Bhagat & Shideman, 1964). To eliminate this endogenous source bilateral adrenalectomy was performed on six cats. In these animals the blood pressure response to adrenaline usually decreased when series of injections were separated by a long interval (Fig. 8). However, infusion of adrenaline could prevent a

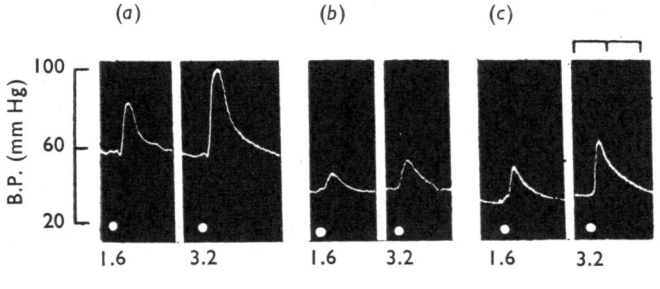

Fig. 8. Effect of time interval and of adrenaline infusion on pressor response to successive applications of adrenaline in the adrenalectomized, spinal cat. (*a*), Responses to adrenaline on first applications; (*b*), responses to second applications, after an interval of 30 min; (*c*), responses after a second interval of 30 min, during which adrenaline, 60 μg/kg, was infused intravenously. Notation as in Fig. 1.

further decrease or even increased somewhat the response to a subsequent single dose of this drug (Fig. 8).

Stimulation of the spinal cord. Discharge of the sympathetic nervous system can result in release of catechol amines from nerve endings and from the adrenal medulla, thus increasing the rate of loss of catechol amines from the organism. It has been recently shown that such depletion increases the capacity of uptake of catechol amines by the heart (Gillis, 1963). Therefore, it was expected that depletion of stores should have an effect opposite to that of loading them (by catechol amine infusions). To this end, electrical stimulation was applied to the stump of the severed spinal cord, producing a generalized discharge of peripheral nerves. In all five experiments of this type the blood pressure response to adrenaline was considerably diminished, as shown in the example of Fig. 9,*a* and *b*. Subsequent infusion of adrenaline restored or even increased the response to the drug (Fig. 9,*c*).

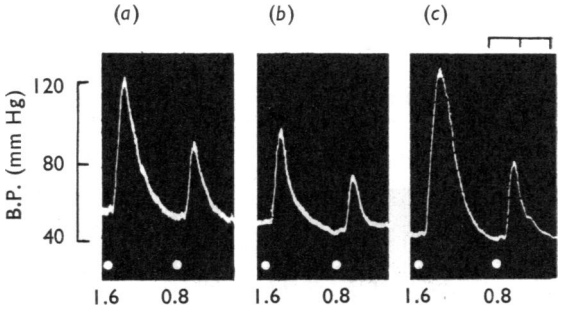

Fig. 9. Effect of spinal cord stimulation and adrenaline infusion on the responses to adrenaline in the spinal cat. (*a*), Before; (*b*), after electrical stimulation of the spinal cord; (*c*), after infusion of adrenaline, 60 μg/kg. Notation as in Fig. 1.

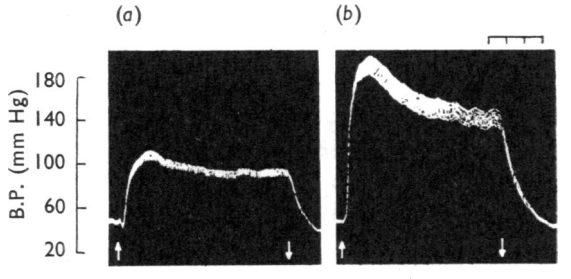

Fig. 10. Effect of cocaine on blood pressure response to adrenaline infusion in the spinal cat. (*a*) and (*b*), First and second intravenous adrenaline infusions, respectively (2 μg/kg/min for 10 min between arrows). Between (*a*) and (*b*), cocaine (2 mg/kg) was injected intravenously. Notation as in Fig. 5.

Cocaine. The influence of cocaine was studied in six animals. Cocaine is well known for its potentiation of the response to catechol amines, intensifying the pressor reaction to infusion of adrenaline. This augmentation, so striking when cocaine was injected between the first and second adrenaline infusion (Fig. 10), was not conspicuous at all if the alkaloid was given after the maximal response to adrenaline had already been attained by repeated infusions of adrenaline (Fig. 11).

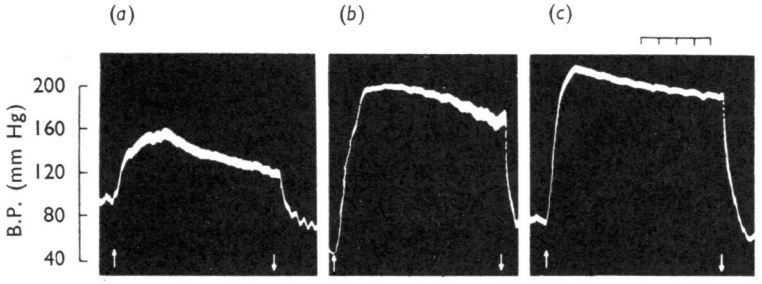

Fig. 11. Effect of repeated adrenaline infusions and cocaine on blood pressure response to adrenaline in the spinal cat. (*a*), (*b*) and (*c*), Intravenous infusions of adrenaline, 2 μg/kg/min for 10 min. Between (*a*) and (*b*), three adrenaline infusions, totalling 60 μg/kg. Between (*b*) and (*c*), cocaine, 2 mg/kg intravenously. Notation as in Fig. 5. Note the considerable augmentation from (*a*) to (*b*), while the effect of cocaine is small compared to that in Fig. 10.

Intra-aortic injections. The increase in response to adrenaline was observed not only upon intravenous administration but also in ten experiments where the drug was repeatedly injected into the thoracic (descending) aorta (Fig. 12). Intra-aortic injection avoids, to

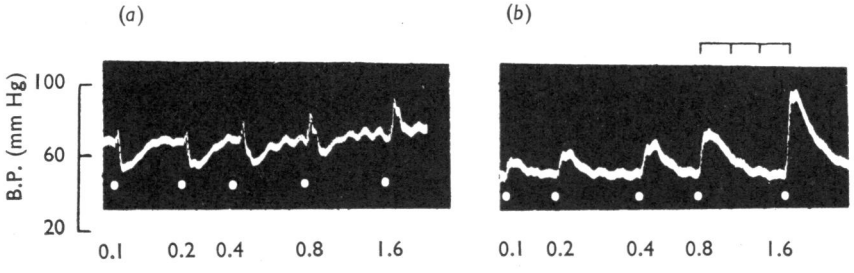

Fig. 12. Effect of adrenaline loading on blood pressure response to intra-aortic injections of adrenaline in the spinal cat. (*a*) and (*b*), Two consecutive series of adrenaline injections into the descending aorta. Between (*a*) and (*b*), repeated intravenous infusions of adrenaline, totalling 100 μg/kg. Notation as in Fig. 1.

a large extent, the participation of cardiac effects in the vasomotor response, as suggested by the lack of change in heart rate in these experiments. The increase in pressor response to intra-aortic injections of adrenaline was equally well observed after adrenaline infusions, given either intravenously or into the aorta.

Effect of large doses of catechol amines. The change in blood pressure response to repeated adrenaline injections, shown in the foregoing experiments, depends on the amount of catechol amines applied during the intervals. Thus, the hypertensive response was considerably enhanced by three infusions of 20 μg/kg during a period of 10 min (Fig. 13,*a* and *b*). On the contrary, infusion of a large dose (220 μg/kg/10 min) of the drug diminished the response to a subsequent single injection (Fig. 13,*c*). The same effect was obtained in four animals infused with large doses of adrenaline. This observation was not peculiar to adrenaline: infusion of a large dose of isoprenaline (2 mg/kg) also resulted in a decrease of the response to adrenaline (Fig. 14), whereas small doses of isoprenaline (20 μg/kg) enhanced the hypertensive reaction to adrenaline (Fig. 2). Similar results were observed in five experiments of this type.

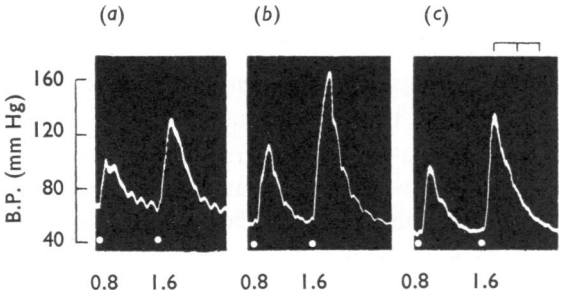

Fig. 13. The inverse effects of small and large infusions of adrenaline on blood pressure response to adrenaline in the spinal cat. Between (*a*) and (*b*), 60 μg/kg; between (*b*) and (*c*), 220 μg/kg of adrenaline infused intravenously. Notation as in Fig. 1.

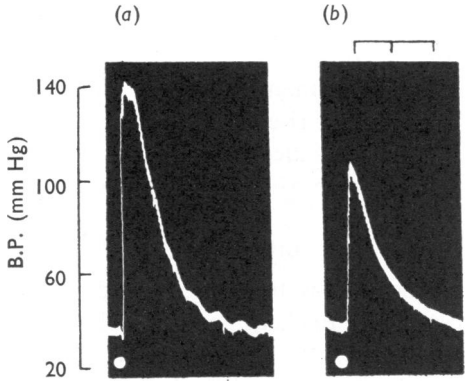

Fig. 14. Effect of a large dose of isoprenaline on blood pressure response to adrenaline in the spinal cat. (*a*) and (*b*), Intravenous injection of 1.6 μg/kg adrenaline at white dots. Between (*a*) and (*b*) isoprenaline, 2 mg/kg, injected intravenously. Notation as in Fig. 1.

After cocaine, depression of the response to adrenaline could be produced by infusion of relatively small doses of the catechol amine. This was studied in six animals. In the example shown in Fig. 15, the response to adrenaline increased after cocaine (*b*), but was diminished by subsequent infusion of only 30 μg/kg of adrenaline (*c*). After an interval of

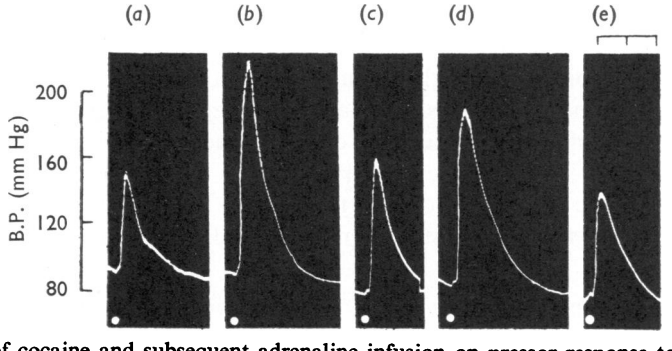

Fig. 15. Effect of cocaine and subsequent adrenaline infusion on pressor response to adrenaline in the spinal cat. At white dots, intravenous injection of 1.6 μg/kg of adrenaline. Between (*a*) and (*b*), cocaine, 5 mg/kg intravenously; between (*b*) and (*c*) and likewise between (*d*) and (*e*), intravenous adrenaline infusion, 30 μg/kg; between (*c*) and (*d*), time interval of 20 min. Notation as in Fig. 1.

20 min some recovery of the response was observed (*d*), but a further infusion of adrenaline (the same as that between (*b*) and (*c*)) resulted again in depression of the reaction to single doses of the drug (*e*). This contrasts with the augmenting effect of infusion of similar doses in the noncocainized animal (Fig. 13,*a* and *b*).

Intact animals. In order to test whether the results hitherto observed applied only to spinal animals, some experiments were carried out in intact cats, anaesthetized with ether. To avoid any effect on blood pressure that could be attributed to changes of muscle tone or respiration, the animals were paralysed by succinylcholine and artificially ventilated. Augmentation of the pressor response to adrenaline and elimination of depressor responses upon repeated administration of the drug were observed in the six animals studied by this method.

Anaesthesia with pentobarbitone. All experiments described above were performed on animals initially anaesthetized with ether, and most of the cats were spinally transected. Both these procedures involve a conspicuous excitatory phase or stress and are therefore accompanied by release of catechol amines. It was expected that, if such effects were avoided, the increase of blood pressure rise following repeated administration of adrenaline would be less prominent. This was indeed observed in five experiments on intact cats, anaesthetized by intramuscular injection of pentobarbitone.

DISCUSSION

The experiments reported here show that repeated administration of catechol amines can lead to an increased response to further injection of these compounds. The augmentation may be due to loading of tissue stores, leading to decreased tissue uptake, by analogy to the increased sensitivity to catechol amines after denervation, when the uptake mechanism is altogether absent (Hertting *et al.*, 1961; Kirpekar *et al.*, 1962). The present experiments also demonstrate that the enhancing effect is shared by four different catechol amines: adrenaline, noradrenaline, isoprenaline and dopamine. Presumably these compounds may use the same storage facilities (Axelrod *et al.*, 1959; Harrison *et al.*, 1963; Rosell, Axelrod & Kopin, 1964; Strömblad & Nickerson, 1961) and prevent competitively each other's uptake. After loading, the various catechol amines, even an unnatural one as α-methylnoradrenaline, can be released from tissues by sympathetic nerve stimulation (Muscholl & Maître, 1963; Rosell *et al.*, 1964). Further support for the role which tissue stores play in the increased response to repeated application of catechol amines is obtained by experiments with cocaine. This alkaloid sensitizes the effector organs to catechol amines and also inhibits uptake of catechol amines by tissues. It has been suggested that these two effects are correlated: inhibition of uptake being the cause of the increased response to catechol amines (Muscholl, 1961; Furchgott, Kirpekar, Rieker & Schwab, 1963). Blockade of uptake thus has the same effect as loading of tissue stores. Consequently, cocaine does not have an enhancing effect after the stores are saturated with catechol amines (compare Figs. 10 and 11).

On the other hand, depletion of the stores, leading to increased rate of uptake by tissues (Siegel *et al.*, 1961; Gillis, 1963), should have an opposite effect. Two procedures, used in the present experiments, namely ether anaesthesia and transection of the spinal cord, cause depletion through sympathetic discharge. In addition, adrenalectomy, performed on some animals, prolonged the depletion by eliminating a major source for restoration

of tissue catechol amines (Bhagat, 1963; Bhagat & Shideman, 1964). (However, interpretation of this type of experiment should be cautious as long as the role of adrenal steroids in catechol amine uptake has not been clarified.) In all these experiments, the reaction to adrenaline could be augmented by infusion of catechol amines. Therefore, it can be concluded that depletion itself reduced the response to catechol amines. Such decrease of the response has been directly demonstrated following stimulation of the spinal cord.

Reserpine is known to deplete tissue stores and to potentiate the pressor response to catechol amines. This combination of actions seems to conflict with the foregoing statements. However, reserpine has been also shown to inhibit the uptake of catechol amines (Dengler, Spiegel & Titus, 1961). Furthermore, the latter phenomenon has been recently shown to correlate better with the pharmacological effects of reserpine than the depletion itself (Lundborg, 1963).

Barbiturate anaesthesia avoids the excitatory stage and, therefore, should not lead to exhaustion of catechol amines stores. Accordingly, in the animal anaesthetized with pentobarbitone, repeated infusions of adrenaline do not produce significant augmentation of the response, as observed in the present study. Interestingly, it has been recently reported that barbiturate can prevent depletion of brain catechol amines induced by electric shocks or cold (Maynert & Levi, 1964).

According to the reasoning developed in the previous sections it is suggested that if a small dose of catechol amine is injected when tissue stores are far from saturation, part of the amine is taken up into stores and only part of it reaches receptor sites. Tissue stores and receptors would thus compete for the catechol amine. By previous loading of tissue stores a larger proportion of a subsequent dose of the amine reaches the receptors, resulting in an increased response. However, if a very large dose of catechol amine is infused so that tissue stores are completely saturated, and a high concentration of the amine is consequently maintained also at receptor sites, a subsequent injection of a single small dose of the amine will be less effective (Fig. 13). Such depressed response to single injections of adrenaline and noradrenaline after large doses had been infused was reported as tachyphylaxis (Rosenthale & DiPalma, 1962; Pérez-Reyes & Lipton, 1963; Pohle & Matthies, 1963). As would be anticipated, this phenomenon is facilitated when tissue uptake is blocked, for example by cocaine (see also Pérez-Reyes & Lipton, 1963). Unfortunately quantitative assessment of the role of tissue stores is not possible because no data are yet available on the storage capacity and turnover rate of catechol amines of the whole animal.

The vasomotor effect of catechol amines is not always pressor. Thus, isoprenaline is known to produce mainly vasodilation. Similarly, adrenaline, especially at low doses, frequently elicits a blood pressure fall. The two opposite effects, blood pressure rise and fall, are ascribed to the action of catechol amines on two different receptors: α- and β-receptors (Ahlquist, 1948). All known catechol amines can act on both types of receptors. For example, isoprenaline can contract spleen strips (Bickerton, 1963), thus exhibiting an α-effect, analogous to pressor responses. Furthermore, after repeated administration, the vasomotor action of isoprenaline changes from depressor into pressor, as reported recently by Butterworth (1963) and confirmed in the present experiments. The assumption that α- and β-effects of catechol amines may be of different nature is also supported by the observation that each action is blocked by different inhibitors (Moran, 1963). It seem that β-effect reaches its maximum while tissue stores of catechol amines are still low;

after loading of the stores, the α-effect becomes dominant, while β-actions are either unchanged (heart rate, Fig. 4), or completely masked (depressor response, Fig. 1). The increasing predominance of α-effects upon loading may be the mechanism underlying the observations of Walz, Koppanyi & Maengwyn-Davies (1960) on the reversal of blood pressure response to isoprenaline after various sympathomimetic amines.

SUMMARY

1. Vasomotor responses to repeated administration of catechol amines were studied in cats anaesthetized with ether and in spinal cats.

2. The pressor response increased upon successive applications of catechol amines, while the tachycardic effect of adrenaline remained unchanged and the depressor reaction disappeared.

3. Small to moderate doses (20 to 60 μg/kg infused over 10 min) of either adrenaline, noradrenaline, dopamine or isoprenaline enhanced the response to subsequent single doses of adrenaline. Barbiturate anaesthesia diminished this enhancement considerably.

4. Potentiation by cocaine was not evident when maximal response to adrenaline had been attained by repeated infusions of the catechol amine.

5. Stimulation of the spinal cord resulted in a decreased response to adrenaline but subsequent infusion of adrenaline augmented again the response to single doses of the drug.

6. Infusion of very large doses of either adrenaline (⩾220 μg/kg) or isoprenaline (2 mg/kg) diminished the pressor response to single doses of adrenaline (" tachyphylaxis "). In the cat treated with cocaine this phenomenon can be produced by small doses of adrenaline (30 μg/kg).

7. The possible mechanism of these phenomena is discussed in relation to inactivation of circulating catechol amines by tissue uptake.

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