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Amphetamine tachyphylaxis in the pithed guinea-pig

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A single dose of (+)-amphetamine (8 mg kg^{-1}, i.p.) administered 4 h before experimentation, reduced the pressor and positive chronotropic effects elicited by this drug (0·6 mg kg^{-1}, i.v.) and augmented the rate of the development of tachyphylaxis to these responses in the pithed guinea-pig preparation. Amphetamine pretreatment reduced the pressor and positive chronotropic effects of phenylephrine (0·1 mg kg^{-1}, i.v.) and the positive chronotropic effects of angiotensin (30 μg kg^{-1}, i.v.). The rate of the development of tachyphylaxis to the cardiovascular responses elicited by phenylephrine and angiotensin was augmented by amphetamine pretreatment. These results suggest that an indirect mechanism (noradrenaline) may in part mediate the cardiovascular effects of these 3 drugs and/or that amphetamine may act as a competitive antagonist at adrenoceptor sites.

When amphetamine is repeatedly administered at short intervals, acute tolerance or tachyphylaxis develops. Tachyphylaxis to the cardiovascular effects of amphetamine has been demonstrated in the anaesthetized dog (Winder, Anderson & Parke, 1948; Eble & Rudzik, 1965), anaesthetized and spinal cat (Day & Rand, 1963; Bhagat, 1965; Day, 1967) and pithed rat (Day,1967). Cross-tachyphylaxis has been shown to develop between amphetamine and phenethylamine (Day & Rand, 1963), but not between amphetamine and other indirectly acting sympathomimetic amines such as tyramine (Bhagat, 1965; Eble & Rudzik, 1965; Day, 1967).

I have examined amphetamine tachyphylaxis in the guinea-pig because information describing this phenomenon is lacking in this species, and *p*-hydroxynorephedrine, a metabolite implicated in the development of amphetamine tolerance in rats, is not formed by guinea-pigs (Costa & Groppetti, 1970). Pretreatment with a single dose of amphetamine was found to reduce the subsequent cardiovascular responses of pithed guinea-pigs to amphetamine, phenylephrine and angiotensin.

METHODS

Male albino guinea-pigs, 400–600 g, were anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.p.), the trachea cannulated, and the animals were pithed by the method of Gillespie & Muir (1967) by passing a rod down the spinal cord and leaving it *in situ*. Respiration was maintained artificially with a Harvard rodent respiratory pump (Model 680). Blood pressure was recorded via the right carotid artery by means of a blood pressure transducer connected to a Grass Model 7 polygraph; heart rate was monitored via a Grass Model 7P4A tachograph. The external jugular vein was cannulated for the administration of drugs.

Before and after each drug injection, 1 ml of saline was administered. Unless otherwise stated, drugs were administered intravenously at 10 min intervals, at which time the blood pressure had approximately returned to pre-drug levels.

Values are expressed as the mean arterial blood pressure (mm Hg) or heart rate (beats min^{-1}) $\pm$ s.e.m., and the data were analysed using Student's *t*-test. The mean arterial blood pressure = diastolic pressure + 1/3 pulse pressure.

Drugs. Angiotensin amide (Ciba Pharmaceutical Co.), (+)-amphetamine sulphate (Smith Kline & French Laboratories), pentobarbitone sodium (Abbott Laboratories) and (−)-phenylephrine hydrochloride (Ganes Chemical Co.). The doses refer to the salt weight of the drugs employed.

RESULTS

Amphetamine tachyphylaxis

No differences were observed between the systolic, diastolic or mean arterial blood pressures of untreated pithed guinea-pigs and comparable animals that had been pretreated 4 h before with saline; the heart rate of saline-pretreated animals was 10% lower than those untreated (Table 1).

Table 1. *Blood pressure and heart rate of untreated, saline and amphetamine pretreated pithed guinea-pigs.*

	Untreated	Saline	Amphetamine
Blood pressure (mm Hg)			
Mean arterial b.p.	34·3 ± 1·0	34·9 ± 1·4	40·5 ± 1·2‡
Systolic b.p.	46·9 ± 1·3	46·0 ± 1·3	52·5 ± 1·2§
Diastolic b.p.	27·8 ± 1·0	29·5 ± 1·4	34·5 ± 1·3†
Heart rate (beats min^{-1})	226·5 ± 6·7	204·1 ± 6·9*	267·1 ± 7·1§

Saline (2 ml kg^{-1}, i.p.) or amphetamine (8 mg kg^{-1}, i.p.) was administered 4 h before monitoring blood pressure and heart rate in pithed guinea-pigs anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.) Values are the mean ± s.e.m. of 17 animals in each group.
* $P < 0.05$ compared with untreated pithed guinea-pigs. † $P < 0.02$, ‡ $P < 0.005$, § $P < 0.001$ greater than corresponding untreated animals and those pretreated with saline.

Administration of six successive injections of amphetamine (0·6 mg kg^{-1}, i.v.) at 10 min intervals elicited a progressively reduced pressor and positive chronotropic effect to this drug. The first injection increased mean arterial blood pressure 15 ± 0·7 mm Hg; injections 2–6 elicited a rise in blood pressure of 8·5 ± 0·4, 5·2 ± 0·5, 4·5 ± 0·3, 3·0 ± 0·7 and 0·3 ± 0·3 mm Hg, respectively. The rise in blood pressure elicited by the first injection was significantly higher ($P < 0.001$) than all subsequent injections (n = 6). Tachyphylaxis developed at a similar rate to the positive chronotropic effects of amphetamine. The first injection increased heart rate 30 ± 3 beats min^{-1}, while injections 2–5 elicited increases of 17·5 ± 2·5, 12·5 ± 2·5, 10 ± 10 and 5 ± 5 beats min^{-1}, respectively; no increase was elicited by the sixth injection. The rise in heart rate produced by dose 1 was greater than dose 2 ($P < 0.025$) and doses 3–6 ($P < 0.01$) (n = 2).

In the next series of experiments, guinea-pigs were pretreated with amphetamine (8 mg kg^{-1}, i.p.) 0·5–48 h before testing the cardiovascular responses of pithed animals to amphetamine (0·6 mg kg^{-1}, i.v.). These experiments were designed to determine the time of intraperitoneal amphetamine-pretreatment that would maximally depress the responses elicited by intravenous administration of this drug. Intraperitoneal injections were administered to pithed animals 0·5 and 1 h before testing, while in all other experiments, pretreatment injections were administered to awakened, intact animals.

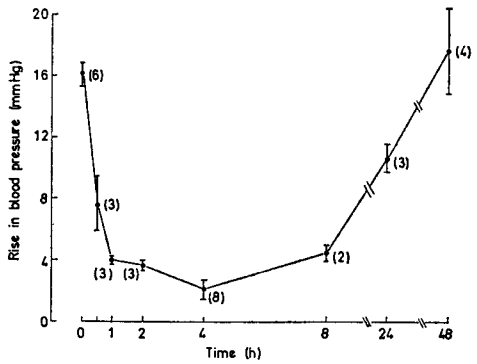

FIG. 1. Influence of the time of (+)-amphetamine (8 mg kg^{-1}, i.p.) pretreatment on the pressor response to amphetamine (0·6 mg kg^{-1}, i.v.). Time 0 represents the response of non-pretreated animals to i.v. amphetamine. The mean response ± s.e.m. is depicted; the figures in parentheses represent the number of animals tested at each pretreatment time.

Intravenous injection of amphetamine to six control non-pretreated pithed guinea-pigs increased mean arterial blood pressure 16 ± 0·9 mm Hg. After intraperitoneal amphetamine pretreatments at 0·5, 1, 2 and 4 h, the average rise in blood pressure was 48, 25, 23 and 13%, respectively, of that observed in control animals (Fig. 1). The systolic and diastolic blood pressures and heart rate of pithed guinea-pigs pretreated 4 h before with amphetamine were 14–17% and 31% higher, respectively, when compared with saline-pretreated animals (Table 1). Eight to 48 h after a single amphetamine injection, the pressor response elicited by intravenous drug administration gradually returned to normal; at 48 h the response was 9% higher than control values. In two control animals, amphetamine (i.v.) increased heart rate 30 ± 0 beats min^{-1} but this positive chronotropic effect was virtually abolished for at least 8 h in pretreated animals; at 4 h no positive chronotropic response was elicited in seven animals and a rise of 5 beats min^{-1} was produced in one animal. Twenty-four h after injection, the chronotropic response to amphetamine (i.v.) returned to normal. From the results obtained in these experiments, it was concluded that a 4 h amphetamine (8 mg kg^{-1}, i.p.) pretreatment schedule would elicit a maximal response and this was employed in all subsequent studies.

In animals pretreated with amphetamine (i.p.), both the initial cardiovascular responses to an intravenous injection of this drug and the total number of injections required to produce tachyphylaxis were reduced; the positive chronotropic response and pressor effects were abolished after two and three injections, respectively (Fig. 2A). By contrast, after the same number of injections in saline-pretreated control animals the initial rise in heart rate and blood pressure was only reduced by 60 and 68%, respectively.

Amphetamine-phenylephrine interactions

Phenylephrine (0·1 mg kg^{-1}, i.v.) increased mean blood pressure and heart rate 62 ± 1·6 mm Hg and 24 ± 2·4 beats min^{-1}, respectively, in saline-pretreated control animals (Table 2–A). A single injection of amphetamine (0·6 mg kg^{-1}, i.v.) administered 10 min before phenylephrine did not modify the pressor response to the latter drug but reduced its positive chronotropic effects by 90% (Table 2–B). Three successive intravenous injections of amphetamine at 10 min intervals reduced phenyl-

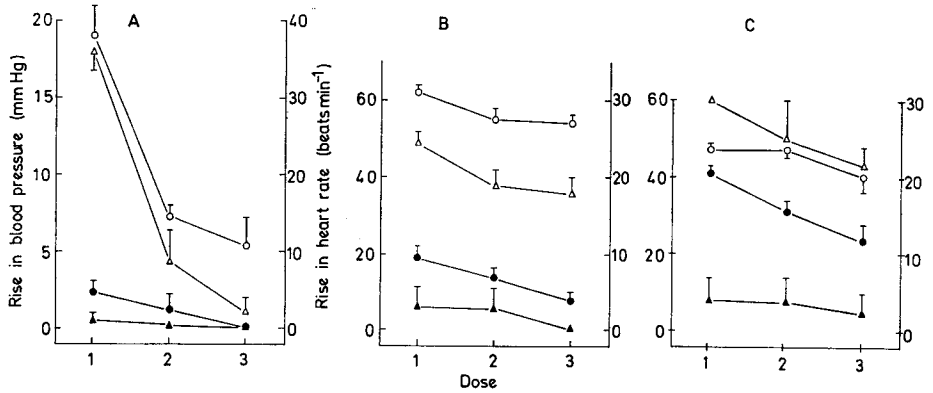

FIG. 2A. Influence of (+)-amphetamine-pretreatment on the rate of the development of amphetamine tachyphylaxis to rise in blood pressure (●) and heart rate (▲). Saline (open symbols) or (+)-amphetamine (8 mg kg^{-1}, i.p., closed symbols) was injected 4 h before testing the response of pithed guinea-pigs to 3 successive doses of (+)-amphetamine (0·6 mg kg^{-1}, i.v.) administered at 10 min intervals. The values represent the mean ± s.e.m. of 5–6 experiments. Saline-pretreated controls, values greater ($P < 0{\cdot}01$) than amphetamine-pretreated animals for rise in blood pressure (doses 1–3) and rise in heart rate (doses 1 and 2) after same number of doses of i.v. amphetamine.

B. Influence of (+)-amphetamine-pretreatment on the rate of the development of phenylephrine tachyphylaxis. Symbols and pretreatment same as A. The values are the mean ± s.e.m. responses to 3 doses of phenylephrine (0·1 mg kg^{-1}, i.v.) administered at 7 min intervals. n = 5–6 experiments. Saline-pretreated controls, values greater ($P < 0{\cdot}001$) than amphetamine-pretreated animals for both rise in blood pressure and heart rate (doses 1–3) after same number of doses of phenylephrine.

C. Influence of (+)-amphetamine-pretreatment on the rate of the development of angiotensin tachyphylaxis. Symbols and pretreatment same as A. The values are the mean ± s.e.m. responses to 3 doses of angiotensin (30 μg kg^{-1}, i.v.) administered at 7 min intervals. n = 3 saline and 6 amphetamine-pretreated animals. Saline-pretreated controls, values greater than amphetamine-pretreated animals for rise in blood pressure (doses 2 and 3, $P < 0{\cdot}025$) and rise in heart rate ($P < 0{\cdot}0025$) after same number of doses of angiotensin.

ephrine's pressor and chronotropic effects by 44 and 79%, respectively (Table 2–C). Amphetamine pretreatment 4 h before phenylephrine administration (in the absence or presence of an intervening intravenous injection of amphetamine), reduced the pressor and positive chronotropic responses to phenylephrine by 55–69% and 86–95%, respectively (Tables 2–D and E).

The cardiovascular responses to three successive injections of phenylephrine were significantly reduced after amphetamine-pretreatment 4 h before the experiment (Fig. 2B). Moreover, the rate of the development of tachyphylaxis to the pressor effects of phenylephrine was augmented in drug-treated animals; the modest positive chronotropic response (3·3 ± 3·3 beats min^{-1}) elicited by the first injection of phenylephrine in these animals was absent by the third injection.

In a preliminary study conducted in four non-pretreated animals, three intravenous injections of phenylephrine at 7 min intervals failed to modify the pressor effects of intravenous amphetamine, but reduced this drug's positive chronotropic response from 36 ± 2·4 to 25 ± 2·9 beats min^{-1} ($P < 0{\cdot}025$).

Amphetamine-angiotensin interactions

Angiotensin (30 μg kg^{-1}, i.v.) raised mean arterial blood pressure and heart rate 43 ± 3·5 mm Hg and 30 ± 0 beats min^{-1}, respectively, in control pithed guinea-pigs. Three successive intravenous injections of amphetamine at 10 min intervals (the last dose given 15 min before angiotensin) reduced the chronotropic effects of the latter

Table 2. *Influence of amphetamine-pretreatment on phenylephrine's cardiovascular effects in the pithed guinea-pig.*

	Pretreatments		Response to phenylephrine (0·1 mg kg^{-1}, i.v.) Rise in blood pressure	Rise in heart rate
Experiment	i.p.	i.v.	mm Hg (n)	beats min^{-1} (n)
A	Saline	Saline	62 ± 1·6 (6)	24 ± 2·4 (5)
B	Saline	Amphetamine (IX)	59 ± 2·6 (3)	2·5 ± 2·5 (2)
C	Saline	Amphetamine (3X)	35 ± 4·2 (5)	5·0 ± 2·5 (4)
D	Amphetamine	Saline	19 ± 3·2 (3)	3·3 ± 3·3 (3)
E	Amphetamine	Amphetamine (3X)	28 ± 5·8 (4)	1·3 ± 1·3 (4)

Saline (2 ml kg^{-1}, i.p.) or amphetamine (8 mg kg^{-1}, i.p.) was administered 3·5–3·75 h before injection of amphetamine (0·6 mg kg^{-1}, i.v.); in experiments C and E, 3 injections of amphetamine were administered at 10 min intervals. The values are the mean ± s.e.m. responses to phenylephrine (0·1 mg kg^{-1}, i.v.).

$P < 0·001$: All amphetamine-pretreatments reduced the pressor response to phenylephrine, in experiments C–E and its chronotropic effects in B–E.

drug by 40% without altering its pressor effects. Angiotensin's pressor and chronotropic effects were reduced 13% ($P < 0·10$) and 86% ($P < 0·001$), respectively, by pretreatment with amphetamine (i.p.) 4 h before testing (Fig. 2C).

In saline-pretreated control animals, the pressor response was non-significantly reduced by 15% after three injections of angiotensin (30 μg kg^{-1}, i.v.) at 7 min intervals (Fig. 2C). Amphetamine-pretreatment (i.p.) significantly reduced angiotensin's pressor response from 41 ± 2·0 mm Hg by 24 and 41% after injections 2 and 3, respectively.

DISCUSSION

Amphetamine is an indirectly acting sympathomimetic amine whose actions have been attributed to release of noradrenaline from storage vesicles in postganglionic neurons and inhibition of the reuptake of this catecholamine after its release. The noradrenaline content of the guinea-pig heart has been shown to be reduced by 35% after the administration of (±)-amphetamine (20 mg kg^{-1}, i.p.) with a slow return to initial values within 24 h (Lewander, 1971). Similar findings have been reported in the same species by Costa & Groppetti (1970) who observed maximum depletion of noradrenaline 1–3 h after (+)-amphetamine (5 mg kg^{-1}, i.p.); concentrations of this catecholamine were elevated at 8 h and almost normal within 24 h. It has been postulated that amphetamine tolerance in rats may result from tissue depletion of noradrenaline and incorporation of the amphetamine metabolite *p*-hydroxynorephedrine into noradrenaline storage granules (Costa & Groppetti, 1970). This mechanism would not explain tolerance to amphetamine in guinea-pigs because this species is incapable of *p*-hydroxylating amphetamine (Dring, Smith & Williams, 1970; Lewander, 1971).

In the present study, the pressor response elicited by amphetamine (i.v.) was most depressed (75–87%) 1–4 h after amphetamine-pretreatment. At 8 and 24 h, the rise in blood pressure was 72 and 34%, respectively, below control values and returned to normal by 48 h (Fig. 1). The positive chronotropic effects were abolished in excess of 8 h after amphetamine-pretreatment and were normal at 24 h. These observations bear close temporal agreement with the biochemical studies of Lewander (1971) and Costa & Groppetti (1970) and suggest that amphetamine depletion of noradrenaline from nerve endings may account for the reduced cardiovascular responses observed in the guinea-pig.

The pithed guinea-pig is less responsive to the pressor effects of amphetamine than is the pithed rat or spinal cat. Rises in the mean blood pressure of 37–51 mm Hg have been reported with doses of 0·09–0·5 mg kg^{-1} in the latter two species (Bhagat, 1965; Day, 1967; Schmitt & Schmitt, 1970). In this study, amphetamine (0·6 mg kg^{-1}, i.v.) elicited a 15–18 mm Hg rise in mean arterial blood pressure. The rate of development of tachyphylaxis to the pressor effects of amphetamine was similar among the three species, notwithstanding differences in dosage and time intervals between injections (Bhagat, 1965; Day, 1967). Further, these experiments indicate that amphetamine-pretreatment, intraperitoneally, reduced the number of injections required to produce complete tachyphylaxis from 6 in control animals to 2–3 (Fig. 2A).

Amphetamine-pretreatment (i.p. and/or i.v.) reduced phenylephrine's pressor and positive chronotropic effects by 44–69% and 79–95%, respectively (Table 2). Moreover, the pressor response elicited by the third injection of phenylephrine in amphetamine (i.p.) pretreated animals was one-half that produced by the first dose (Fig. 2B).

Phenylephrine-amphetamine interactions may be mediated indirectly by noradrenaline. While high doses of phenylephrine (5 mg kg^{-1}, s.c.) enhance noradrenaline release from the mouse heart (Daly, Creveling & Witkop, 1966), the weight of literature fails to support an indirect (noradrenaline-mediated) component responsible for phenylephrine's vasopressor and positive chronotropic effects (Burn & Rand, 1958; Trendelenburg, Gomez Aconso de la Sierra & Muskus, 1963). Amphetamine may reduce phenylephrine's effects by occupying the adrenoceptor site and thereby act as a competitive antagonist (Furchgott, 1970).

A large body of evidence suggests that adrenergic interactions mediate the cardiovascular effects of angiotensin (McCubbin, 1974; Regoli, Park & Rioux, 1974), yet conflicting experimental observations have complicated the formulation of a unified underlying mechanism. The pressor effects of angiotensin have been reported to be reduced or unchanged in the pithed rat; desipramine or reserpine did not alter or potentiate these pressor effects and tyramine enhanced the rise in blood pressure (Schmitt & Schmitt, 1968; Day & Owen, 1969; Finch & Leach, 1969). In this study, amphetamine-pretreatment did not significantly modify the rise in blood pressure produced by the first injection of a large dose of angiotensin, but augmented the rate of the development of tachyphylaxis; the pressor responses elicited by the third dose of angiotensin in amphetamine and saline-pretreated animals were 41 and 14%, respectively, lower than produced by the first dose (Fig. 2C). Angiotensin's positive chronotropic effects were present in adrenalectomized, pithed rats, yet were abolished by reserpine, bethanidine (an adrenergic neuron blocker) and propranolol (Finch & Leach, 1969). These investigators have postulated that the chronotropic effects of angiotensin result from adrenergic stimulation at the ganglia or nerve endings. Amphetamine-pretreatment reduced the peptide induced rise in heart rate by 40–86%. This diminished response might be the consequence of noradrenaline depletion from synaptic granules by amphetamine.

Amphetamine-pretreatment intraperitoneally significantly reduced the positive chronotropic response to intravenous administration of amphetamine (98%), phenylephrine (86%) and angiotensin (86%) in pithed guinea-pigs. The results suggest that all three drugs may have an indirect component associated with their positive chronotropic effects and/or that amphetamine may act as a competitive antagonist at the adrenoceptor site after its administration.

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