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An analysis of central adrenoceptors for control of cardiovascular function

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Summary

1. In dogs anaesthetized with pentobarbitone sodium, bradycardia with hypotension occurred on intracerebroventricular (i.c.v.) injection of noradrenaline (50–200 μg) or phenylephrine (100–400 μg), but tachycardia with hypotension occurred on i.c.v. injection of isoprenaline (100–200 μg).

2. These cardiovascular responses were central effects, and from the results obtained after bilateral vagotomy, removal of both stellate ganglia and transection of the upper cervical cord, it was evident that the efferent nervous pathway for all these effects was the sympathetic nervous system.

3. An i.c.v. injection of the α-adrenoceptor blocking agent phenoxybenzamine (10 mg) blocked the bradycardia and hypotension produced by noradrenaline or phenylephrine, and an i.c.v. injection of a β-adrenoceptor blocking agent, either propranolol (2 mg) or *N*-isopropyl-*p*-nitrophenyl-ethanolamine (INPEA) (10 mg), blocked the tachycardia and hypotension produced by isoprenaline.

4. The cardiovascular effects produced by i.c.v. injection of the three sympathomimetic amines could be reproduced in cross-circulation experiments in the recipient dog when the amines were injected into its head circulation, and the effects of noradrenaline and phenylephrine, but not those of isoprenaline, were abolished by the α-adrenoceptor blocking agent yohimbine (1 mg/kg) injected intravenously into the donor dog.

5. It is concluded that the central α-adrenoceptors are concerned with bradycardia and the central β-adrenoceptors with tachycardia, but that both receptors are concerned with hypotension.

Introduction

Catecholamines and the enzymes responsible for their biosynthesis and inactivation have been localized in several brain areas (Vogt, 1954 ; Udenfriend & Creveling, 1959 ; Weiner, 1960), and adrenergic neurones and nerve tracts have been demonstrated in the central nervous system with the use of the fluorescence microscopic technique (Carlsson, Falck & Hillarp, 1962 ; Dahlstrom & Fuxe, 1964). For the transmitter of adrenergic neurones in the sympathetic nervous system, two types of receptors have been postulated by Ahlquist (1948), the alpha and beta receptors. In the central nervous system these two types of adrenoceptors have been considered for a few functions only, for the control of behaviour, electrocortical activity and ovulation (Goldstein & Munoz, 1961 ; Gupta, Gupta & Bhargava, 1968).

Central adrenoceptors appear also to be involved in cardiovascular regulation as suggested by the following observations. Intracerebroventricular (i.c.v.) injections of noradrenaline or adrenaline were found to produce bradycardia and hypotension in cats (Nashold, Mannarino & Wunderlich, 1962 ; Share & Melville, 1963 ; Gagnon & Melville, 1966) as well as in dogs (McCubbin, Kaneko & Page, 1960 ; Srimal, 1962), and i.c.v. injections of isoprenaline to produce tachycardia and hypotension in cats (Gagnon & Melville, 1967). Further, the cardiovascular responses produced in dogs by i.c.v. noradrenaline were found to be abolished by i.c.v. phentolamine (Kaneko, McCubbin & Page, 1960), and those produced by i.c.v. isoprenaline to be prevented by i.c.v. propranolol and pronethanol (Gagnon & Melville, 1967).

The present investigation is concerned with the nature of the central adrenoceptors involved in cardiovascular functions. Central effects on heart rate and blood pressure were produced in anaesthetized dogs with three sympathomimetic amines, noradrenaline, phenylephrine and isoprenaline ; the influence of α- and β-adrenoceptor blocking agents on these effects was then examined. To obtain the central cardiovascular effects of the three sympathomimetic amines without any interference from their peripheral effects, the amines were given either by i.c.v. injection or, in cross-circulation experiments, by intracarotid injection into the isolated head circulation of the recipient dog.

Methods

Sixty-two mongrel dogs of either sex weighing between 5 and 16 kg were used. They were anaesthetized with intravenous pentobarbitone sodium (30 mg/kg). After cannulation of the trachea, positive pressure artificial ventilation was begun and maintained throughout the experiment. One femoral vein was cannulated with a polythene tube for intravenous injections. In most experiments the blood pressure was recorded from a femoral artery on a Sanborn 150 polygraph with a Statham P23 transducer, and to determine the heart rate the electrocardiogram (Lead II) was taken on another channel of the polygraph. In a few experiments the blood pressure was recorded from a common carotid artery with a mercury manometer on smoked kymograph paper. For i.c.v. injections, one lateral cerebral ventricle was cannulated as described by Bhargava & Tangri (1959). The volume of fluid used for the i.c.v. injection of drugs was 0·25 ml.

To find out if the efferent pathway for the cardiovascular responses produced by the i.c.v. injections of the sympathetic amines was the vagus or the sympathetic, three surgical procedures of interrupting nervous pathways were used. (1) Bilateral vagotomy in the neck. (2) Transverse section of the spinal cord at C_2. (3) Bilateral ganglionectomy of the stellate ganglia. For this purpose the sympathetic chain up to the third and fourth thoracic ganglia was exposed through an incision in the third intercostal space, and the stellate ganglion was removed on each side with its branches and a 3 cm long piece of the sympathetic chain including the 4th and 5th thoracic ganglia.

Cross circulation experiments. The method of isolating the head circulation of the recipient dog and establishing cross circulation between the donor and recipient dog was that described by Taylor & Page (1951). The donor dog was 2 to 4 kg heavier than the recipient in which the extradural venous sinuses were occluded with a No. 8 piano wire. The wire had a tygon covering in its middle part which fitted snugly on the sinus and was tied firmly around the inter-

vertebral disc between the 4th and 5th cervical vertebrae with the help of a tonsil snare. In addition, the vertebral arteries were ligated at their origin in the neck. The completeness of vascular isolation of the head of the recipient was established by failure of a pressure response in its body on intracarotid injection of noradrenaline (5 μg/kg) and by failure of Evan's blue to appear in its body when the dye was injected into the head circulation.

Drugs used. (−)-Noradrenaline hydrochloride, (−)-phenylephrine hydrochloride, (−)-isoprenaline sulphate, phenoxybenzamine hydrochloride, yohimbine hydrochloride, (±)-propranolol hydrochloride and *N*-isopropyl-*p*-nitrophenyl ethanolamine hydrochloride (INPEA). All doses refer to the salts.

Results

Experiments with i.c.v. injections

Noradrenaline produced bradycardia and hypotension on i.c.v. injection. The bradycardia produced by 50, 100 and 200 μg was dose-dependent, but not the hypotension which was never greater than 15 mmHg with these doses. A typical experiment is illustrated in Fig. 1, and in Fig. 2A, the mean changes produced in 11 dogs are given. The mean decreases in heart rate were 11·2, 25·2 and 53 beats per min respectively with the three doses. Neither the bradycardia nor the hypotension occurred when noradrenaline (100 μg) was injected 1 h after an i.c.v. injection of 10 mg of the α-adrenoceptor blocking agent phenoxybenzamine.

Phenylephrine acted like noradrenaline. The bradycardia produced by an i.c.v. injection of 100, 200 and 400 μg phenylephrine was dose-dependent, but not the

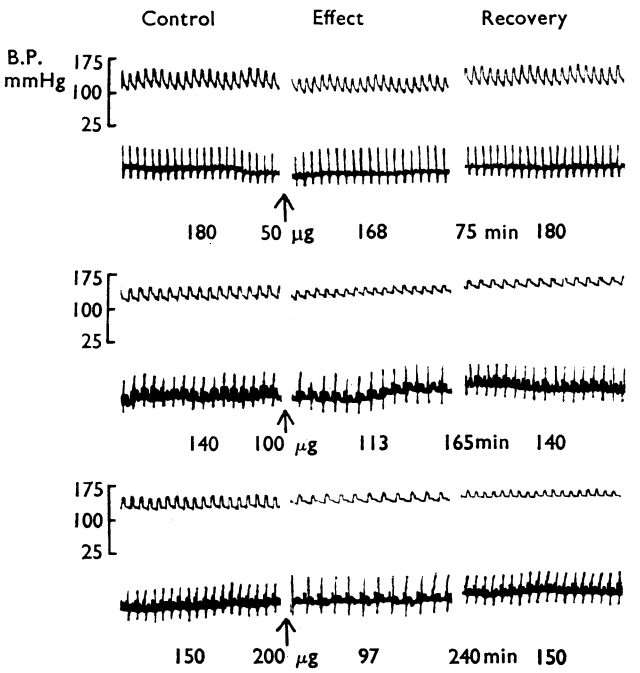

FIG. 1. Arterial blood pressure and electrocardiogram (E.C.G.) from an 8 kg dog anaesthetized with pentobarbitone sodium. At the arrows, i.c.v. injections of noradrenaline. The figures below the E.C.G. records refer to heart rate per min, μg of noradrenaline injected and minutes after injection.

hypotension, which was never more than 15 mmHg with these doses. The mean changes produced in 10 dogs are given in Fig. 2B. The mean decreases in heart rate were 13·8, 27·7 and 53 beats per min respectively with these three doses. No bradycardia and no hypotension occurred when phenylephrine (100 μg) was injected 1 h after an i.c.v. injection of 10 mg of phenoxybenzamine.

Isoprenaline produced tachycardia and hypotension on i.c.v. injection. The mean changes produced in 10 dogs with 100 and 200 μg are given in Fig. 2C. The mean increase in heart rate amounted to 12 and 25·4 beats per min, and the mean fall in blood pressure to 11·4 and 22·6 mmHg respectively with these two doses.

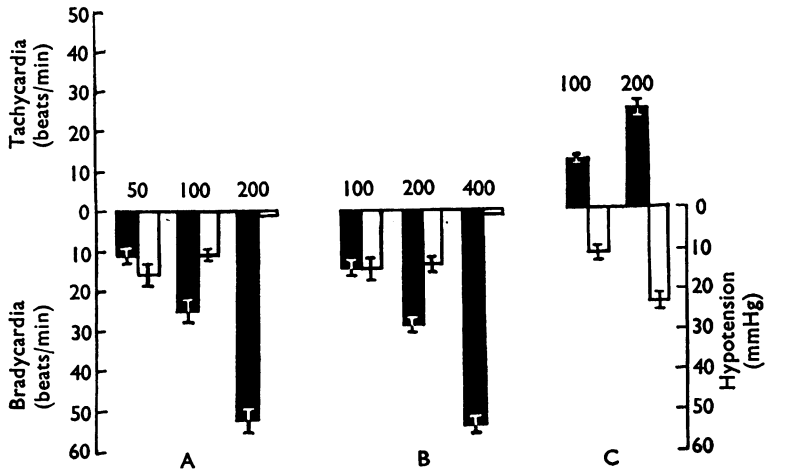

FIG. 2. Histograms giving the mean changes in heart rate and arterial blood pressure produced under pentobarbitone sodium anaesthesia (A) in 11 dogs with i.c.v. injection of 50, 100 and 200 μg noradrenaline, (B) in 10 dogs with i.c.v. injections of 100, 200 and 400 μg phenylephrine and (C) in 10 dogs with i.c.v. injections of 100 and 200 μg of isoprenaline. ■, Heart rate. □, Blood pressure.

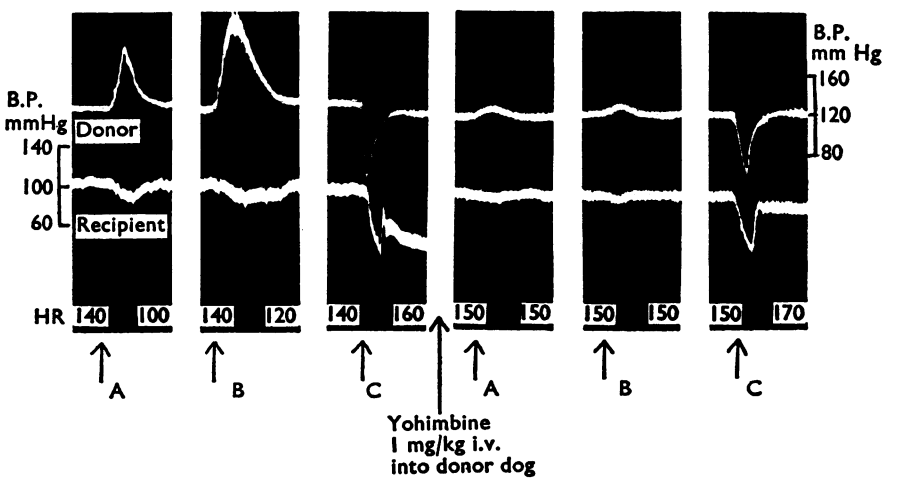

FIG. 3. Arterial blood pressure of recipient 10 kg dog (lower record) and donor 14 kg dog (upper record) anaesthetized with pentobarbitone sodium in cross-circulation experiment. Intracarotid injections into recipient of 100 μg noradrenaline (at A) 1 mg phenylephrine (at B) and 20 μg of isoprenaline (at C). The arrow between the third and fourth panel indicates intravenous injection into donor dog of 1 mg/kg yohimbine. HR=heart beats per minute.

When an injection of isoprenaline (200 μg) was made 1 h after an i.c.v. injection of a β-adrenoceptor blocking agent, either propranolol (2 mg) or INPEA (10 mg), tachycardia and hypotension no longer occurred.

Surgical interruption of nervous pathways

Table 1 summarizes the results with i.c.v. injection of 100 μg noradrenaline, phenylephrine or isoprenaline obtained after cutting the vagi, removal of the stellate ganglia and transection of the cervical cord. From the Table it is evident that bilateral vagotomy did not prevent or affect the cardiovascular responses to any of the three sympathomimetic amines, but that bilateral removal of the stellate ganglia prevented the bradycardia produced by noradrenaline and phenylephrine, the tachycardia produced by isoprenaline and the hypotension produced by phenylephrine but not the hypotension produced by either noradrenaline or isoprenaline. However, all the cardiovascular responses to the i.c.v. injections of the amines, including the hypotension produced by noradrenaline and isoprenaline were prevented by transection of the spinal cord at C_2.

Cross circulation experiments

The cardiovascular effects of intracarotid injection of noradrenaline, phenylephrine and isoprenaline into the recipient dog were the same as those observed with i.c.v. injections. Phenylephrine was less and isoprenaline more potent than noradrenaline. The intracarotid injection of noradrenaline in a dose of 100 μg resulted in bradycardia of 30 to 40 beats per min and hypotension of 10 to 15 mmHg in the recipient dog and in a rise of blood pressure in the donor dog. An intracarotid injection of phenylephrine in doses of up to 500 μg did not affect heart rate or blood pressure in the recipient dog, but the intracarotid injection of 1 mg resulted in bradycardia of 15 to 20 beats per min and in hypotension of 10 to 15 mmHg in the recipient dog along with a pressor response in the donor dog. An intracarotid injection of 20 μg isoprenaline was sufficient to produce a tachycardia of 20 to 30 beats per min and a hypotension of 30 to 40 mmHg in the

TABLE 1. *Effect of surgical interruption of nervous pathways on the cardiovascular effects of i.c.v. sympathomimetic amines*

Drugs	Change in heart rate (beats/min) Mean±S.E.	*P* value	Fall in blood pressure (mmHg) Mean±S.E.	*P* value
Noradrenaline (100 μg)				
Normal	(−) 25·2±1·8	—	11·25±1·2	—
Vagotomized	(−) 22·8±1·6	>0·05	11·0 ±1·0	>0·05
Stellate ganglionectomy	No change	—	11·7 ±1·0	>0·05
Spinal section (C_2)	No change	—	No change	—
Phenylephrine (100 μg)				
Normal	(−) 13·8±1·4	—	13·8 ±2·4	—
Vagotomized	(−) 13·6±1·1	>0·05	15·0 ±2·2	>0·05
Stellate ganglionectomy	No change	—	No change	—
Spinal section (C_2)	No change	—	No change	—
Isoprenaline (100 μg)				
Normal	(+) 12·0±0·5	—	11·4 ±1·0	—
Vagotomized	(+) 13·0±0·9	>0·05	12·8 ±1·0	>0·05
Stellate ganglionectomy	No change	—	12·0 ±1·0	>0·05
Spinal section (C_2)	No change	—	No change	—

(+)=Increase. (−)=Decrease.

recipient dog and a depressor effect in the donor dog. The effects of such injections of the three amines are shown in the experiment of Fig. 3 in the first three panels at A, B and C. When the same intracarotid injections were repeated after an intravenous injection of the α-adrenoceptor blocking agent yohimbine (1 mg/kg) into the donor dog, the cardiovascular effects of noradrenaline and phenylephrine in the recipient and donor dog were no longer obtained, but those of isoprenaline were unaffected. This is shown in the last three panels at A, B, and C of Fig. 3.

Discussion

The cardiovascular responses to i.c.v. injections of noradrenaline, phenylephrine and isoprenaline, in anaesthetized dogs were central effects and could be reproduced in cross-circulation experiments when the amines were injected into the head circulation of the recipient dog. The sympathetic nervous system was found to be the efferent pathway and not the vagi, because the effects still occurred after bilateral vagotomy. On the other hand, after removal of both stellate ganglia neither the bradycardia in response to i.c.v. noradrenaline or phenylephrine nor the tachycardia in response to i.c.v. isoprenaline could be obtained. Removal of the stellate ganglia also prevented the hypotension produced by phenylephrine but not that produced by the noradrenaline or isoprenaline injections (see Table 1). To abolish all the cardiovascular responses to the i.c.v. injection including the hypotension to noradrenaline and isoprenaline required transection of the cervical cord. This suggests that inhibition of sympathetic vasomotor tone in the lower half of the body plays a more prominent role in the hypotension produced by i.c.v. noradrenaline and isoprenaline than by i.c.v. phenylephrine.

The finding that α-adrenoceptor blocking agents blocked the bradycardia produced by noradrenaline and phenylephrine, and β-blocking agents the tachycardia produced by isoprenaline, suggests that activation of central α-receptors is the cause of bradycardia, and activation of central β-receptors is that of tachycardia. On the other hand, inhibition of sympathetic vasomotor tone appears to be brought about by an action on both these receptors, since the hypotension produced by noradrenaline or phenylephrine was abolished by α-adrenoceptor blocking agents and that produced by isoprenaline by β-adrenoceptor blocking agents.

These suggestions are supported by results obtained with other drugs. The bradycardia induced by catapresan injected into the cisterna magna was attributed to activation of central α-adrenoceptors since it was blocked by piperoxane similarly injected (Schmitt, Schmitt & Fenard, 1971), whereas the tachycardia produced by i.c.v. hydrallazine appeared to be due to central α-adrenoceptor blockade (Gupta & Bhargava, 1965). According to Share & Melville (1965a), brain stem noradrenaline is involved in the activation of the sympathetic stimulation produced by picrotoxin and tyramine, and the picrotoxin induced tachycardia was found to be blocked by i.c.v. dichloroisoproterenol (Share & Melville, 1965b). Similarly, the tachycardia and cardiac arrhythmia produced by i.c.v. aconitine (Bhargava, Kohli, Sinha & Tayal, 1969) were blocked by i.c.v. injection of β-blocking agents (Bhargava & Sinha, 1969; Bhargava & Srivastava, 1972a). Blockade of central β-adrenoceptors may also be the cause of the bradycardia produced by i.c.v. propranolol (Bhargava & Srivastava, 1972b). On the other hand, the α-adrenoceptor blocking agent piperoxane, injected intracisternally was shown to block the central vasodepressor effect of catapresan (Schmitt *et al.*, 1971) and the β-adrenoceptor

blocking agent pronethalol, injected i.c.v., to block the hypotension of i.c.v. guanethidine. Its hypotensive effect was mediated by the catecholamines in the brain because it no longer occurred when these stores were depleted (Bhargava, Jaju & Tangri, 1966).

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