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AN INVESTIGATION OF THE TACHYCARDIA PRODUCED BY INTRACEREBRO-VENTRICULAR INJECTIONS OF ISOPRENALINE IN MICE

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1 Isoprenaline, 3.5-20 ng, injected intracerebroventricularly in atropinized mice under pentobarbitone anaesthesia produced a dose-dependent tachycardia.

2 Pretreatment with either reserpine or pempidine blocked nervously-mediated tachycardia as shown by marked reduction of that due to stimulation of the spinal outflow in pithed mice. After pretreatment with these drugs, intracerebroventricular isoprenaline caused tachycardia of a similar degree and time course to that in mice not so pretreated.

3 Pretreatment with either reserpine or pempidine caused supersensitivity to the tachycardia due to intravenous isoprenaline.

4 When allowance was made for this supersensitivity in the effect of intracerebroventricular isoprenaline in pretreated mice, a small dose-dependent residual effect remained that could be attributed to leakage of isoprenaline into the peripheral circulation.

5 This was confirmed by the appearance of a late-developing tachycardia on intracerebroventricular injection of isoprenaline in spinal mice.

6 It is therefore concluded that the tachycardia caused by intracerebroventricular isoprenaline in mice is, at least initially, of central origin.

Introduction

Evidence suggesting the existence of central adrenoceptors has been available since 1960, when Page and his co-workers (McCubbin, Kaneko & Page, 1960; Kaneko, McCubbin & Page, 1960) showed that intracerebroventricular noradrenaline caused cardiovascular effects susceptible to α-adrenoceptor blockade, though these authors suggested that these were indirectly due to vascular changes. Share & Melville (1964) demonstrated that injection of picrotoxin into the lateral cerebral ventricle of the cat induces sympathetic stimulation, producing cardiovascular changes mediated via a release of brain stem noradrenaline. In later experiments (Share & Melville, 1965), it was shown that while the hypotension observed after picrotoxin injection could be abolished by intracerebroventricular phenoxybenzamine, the tachycardia remained unaffected. Intracerebroventricular dichloro-isoprenaline reduced the tachycardia, while the hypotension remained unchanged.

Intracerebroventricular injection of isoprenaline in anaesthetized cats has been shown to produce tachycardia and hypotension, these effects being abolished by pretreatment with pronethanol or propranolol by the same route (Gagnon & Melville, 1967). From this evidence it was suggested that sympathetic receptors exist in central regions paralleling those postulated in the periphery by Ahlquist (1948).

Bhargava, Mishra & Tangri (1972) have shown these effects of intracerebroventricular isoprenaline to be central in origin in anaesthetized dogs by experiments in which bilateral removal of the stellate ganglion or transection of the upper spinal cord was carried out. Using conscious cats Day & Roach (1972) showed that intracerebroventricular isoprenaline produced tachycardia and hypertension, the latter being due to an increased systolic pressure. These effects could be blocked by peripheral injection of pempidine or intracerebroventricular injection of propranolol.

Intracerebroventricular injection of agents in mice (Haley & McCormick, 1957; Brittain, 1966) can be followed by leakage into the general circulation to an extent greater than that indicated by the injection of opaque materials (Shaw, 1974), leading to otherwise unsuspected systemic effects. Cowell & Davey (1968) have shown that the

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effects of intracerebroventricularly injected noradrenaline are mediated partly by a fraction that leaks to the periphery.

In the present studies, we have investigated the possibility that the effects of intracerebroventricularly injected isoprenaline in the mouse may be produced in this way, rather than by direct interaction with central β-adrenoceptors.

Method

Male CFW mice were anaesthetized with sodium pentobarbitone, 100 mg/kg intraperitoneally, 15 min after pretreatment with atropine methylbromide, 2 mg/kg subcutaneously. Each animal was placed on a warming pad and electrodes were inserted in the skin of the chest wall to record the ECG. From this the heart rate was measured using an ECG preamplifier and a Devices ratemeter.

Fifteen min after the pentobarbitone, the control heart rate was read for each mouse and isoprenaline in a volume of 10 μl of solution was injected intracerebroventricularly, in a manner similar to that described by Haley & McCormick (1957). A 0.5 ml Hamilton syringe with a No. 20 needle, 3 mm long was used. The needle was inserted vertically through a point in the skull on the parieto-frontal suture. Examination after the injection of ink showed material in the lateral and third ventricles.

Control animals were injected with 0.9% w/v NaCl solution (saline) intracerebroventricularly and were included in each experiment. All solutions injected intracerebroventricularly were warmed to 37°C before administration. Readings of heart rate were taken at 1, 3, 5, 7, 10, 12 and 15 min after injection.

Some animals were pretreated with reserpine, 3 mg/kg intraperitoneally, 18 h before use, while others received pempidine, 5 mg/kg intraperitoneally, 30 min before use.

In some experiments, mice were pithed and the spinal outflow stimulated electrically by the method of Gillespie & Muir (1967). These animals were pithed with a blunted serum needle inserted through the orbit after cannulation of the trachea. This was carried out under ether anaesthesia after pretreatment with atropine methylbromide.

The tracheal cannula was then connected to a Palmer respiration pump and the animal respired at 200 strokes/min at a pressure of 10 cm water. The animals were then bilaterally adrenalectomized. The spinal outflow was stimulated electrically, through a needle placed in a hind limb to act as an indifferent electrode and with a Grass stimulator connected to the pithing rod to deliver, for 15 s, a square wave stimulation of 10 Hz, 2 ms pulse width and variable voltage; pretreatment with (+)-tubocurarine, 2 mg/kg intraperitoneally, was given to reduce skeletal muscle twitches.

In later experiments, spinal mouse preparations were used. The animals were again pretreated with atropine methylbromide, (2 mg/kg s.c.), and given pentobarbitone, (100 mg/kg i.p.). Ten min later, a tracheal cannula was inserted and a loop of nylon line drawn round the spinal column, ensuring that the carotid arteries were not included. By tightening the loop, severance of the spinal cord occurred, as judged by the cessation of respiration. These animals were then artificially respired as described earlier. Each animal was allowed 15 min to equilibrate after spinalization and either isoprenaline or saline was then injected intracerebroventricularly, the heart rate being measured as previously described. For these experiments, intact control animals were used, pretreated with atropine methylbromide and pentobarbitone and left for 30 min before being injected with saline or isoprenaline intracerebroventricularly.

Drugs used were: atropine methylbromide (IC Pharmaceuticals), pentobarbitone sodium (Abbott), isoprenaline sulphate B.P. (Burroughs Wellcome), (+)-tubocurarine chloride (Burroughs Wellcome), reserpine (Ciba), pempidine tartrate (May & Baker).

Results

Effect of intracerebroventricular isoprenaline

After pretreatment with atropine methylbromide, the effect of pentobarbitone anaesthesia was to produce a gradual decline in the resting heart rates of mice over 25 min (Figure 1). After injecting saline intracerebroventricularly there was a further, more abrupt fall in heart rate which lasted for 6-7 min (Figure 1). This phenomenon was also observed after intracerebroventricular introduction of a 'dry needle'; thus the abrupt fall in heart rate observed with saline was a consequence of the injection procedure.

The effect of an intracerebroventricular injection of isoprenaline was a dose-dependent tachycardia followed by a gradual decline, despite the bradycardia produced by the act of injection. The net effects of the intracerebroventricular isoprenaline were calculated as the difference from control animals similarly injected with saline (ΔHR, Figure 2).

Effect of pretreatment with agents blocking the sympathetic nervous system

In order to determine the peripheral contribution to tachycardia from any amine leaking into the

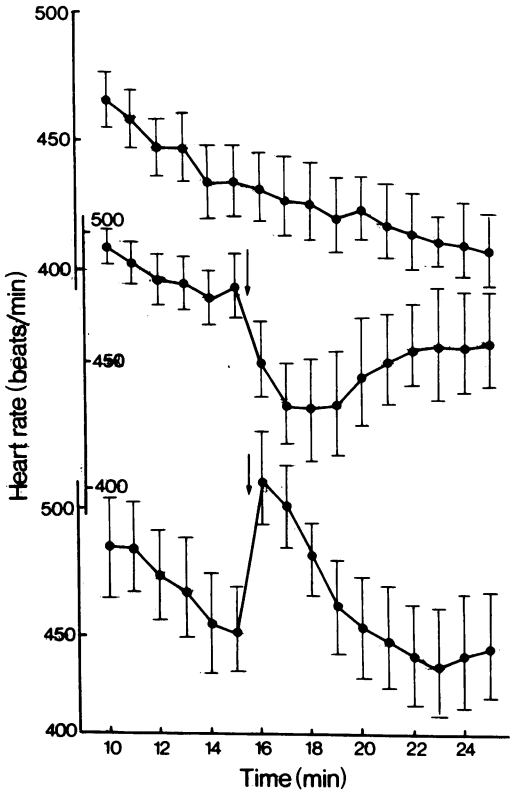

Figure 1 Mean heart rate of groups of mice, read at 1 min intervals from 10 min after injection with pentobarbitone sodium, (100 mg/kg i.p.); 15 min before this they had received atropine methylbromide (2 mg/kg s.c.). From above downwards: no further treatment; intracerebroventricular injection of saline, 10 μl, at arrow; intracerebroventricular injection of isoprenaline, 10 ng in 10 μl, at arrow. Vertical lines indicate s.e. mean.

general circulation, mice were pretreated with reserpine, (3 mg/kg i.p.) 18 h before the intracerebroventricular isoprenaline administration, to block the central component of the effect. This reduced the resting heart rate from 414.3 ± 8.3 to 243.8 ± 11.2 beats per minute.

After this pretreatment, intracerebroventricular isoprenaline produced a dose-dependent tachycardia very similar to that observed in non-reserpine-treated animals (Figure 3). These results can usefully be compared by plotting against dose the sum of ΔHR for all readings taken over 15 min after injection. ($\Sigma\Delta HR = \Delta HR_1 + \Delta HR_3 + \Delta HR_5 + \Delta HR_7 + \Delta HR_{10} + \Delta HR_{12} + \Delta HR_{15}$). Figure 4 suggests that reserpine pretreatment increased rather than reduced the degree of tachycardia due to intracerebroventricular isoprenaline administration.

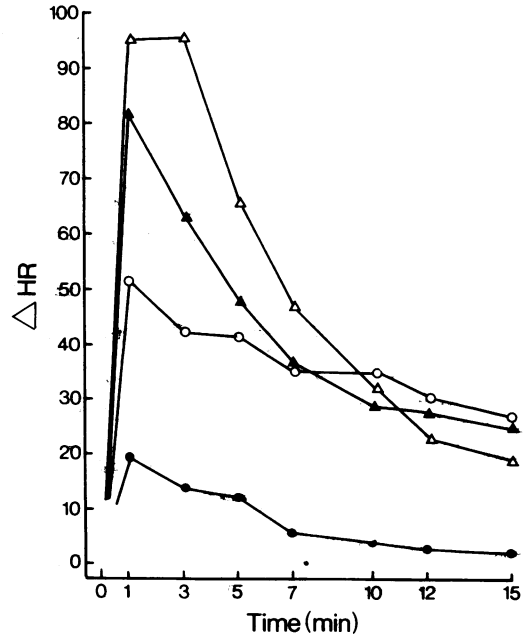

Figure 2 The effect upon the mean heart rate of groups of mice of various doses of isoprenaline injected intracerebroventricularly after pretreatment with atropine methylbromide and anaesthesia with pentobarbitone sodium, as in Figure 1. ΔHR = mean of difference between individual values and mean for control group, injected intracerebroventricularly with saline, read at the same time. Isoprenaline (●) 3.5 ng (n = 49); (○) 5 ng (n = 49); (▲) 10 ng (n = 50); (△) 20 ng (n = 25).

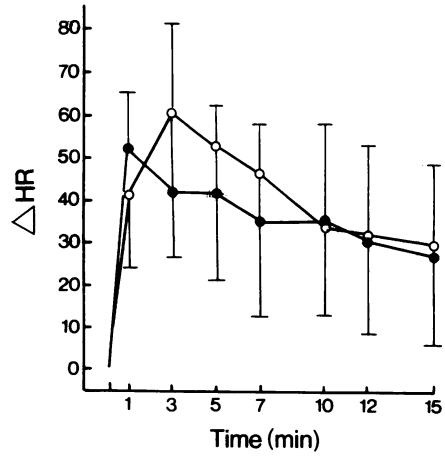

Figure 3 The effect upon heart rate of 5 ng isoprenaline, injected intracerebroventricularly in groups of mice anaesthetized with pentobarbitone sodium and pretreated with atropine methylbromide, with (○) or without (●) additional pretreatment with reserpine (3 mg/kg i.p.) 18 h earlier.

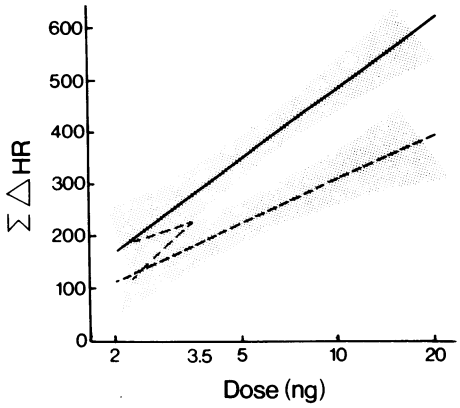

Figure 4 Dose-response relations for the effect upon heart rate of intracerebroventricularly injected isoprenaline in groups of mice anaesthetized with pentobarbitone sodium and pretreated with atropine methylbromide, with (unbroken line) or without (broken line) additional pretreatment with reserpine, as in Figure 3.
$\Sigma\Delta$ HR = Δ HR_1 + Δ HR_3 + Δ HR_5 + Δ HR_7 + Δ HR_{10} + Δ HR_{12} + Δ HR_{15}, where subscripts denote minutes after i.c.v. injection.
The shaded areas denote the 95% confidence limits. For reserpine-treated mice n = 119; for mice not treated with reserpine n = 198.

Isoprenaline injected intravenously into reserpine-pretreated mice was found to produce an even greater tachycardia than in non-reserpine-treated animals (Figure 5). This may be seen by the displaced relation between dose and response in terms of $\Sigma\Delta$ HR when the increase in heart rate due to intravenous isoprenaline in normally sensitive mice was plotted against that in mice rendered supersensitive by pretreatment with reserpine (Figure 6). It appeared that levels of circulating isoprenaline that normally cause a measurable tachycardia caused a greater tachycardia in reserpine-treated mice by a fixed amount (ΔHR = 63.5 beats/min) at all time intervals; normally sub-threshold levels of circulating isoprenaline cause tachycardia of less than ΔHR = 63.5 beats/min in reserpine-treated mice.

As an alternative means of blocking the central components, mice were pretreated with pempidine, (5 mg/kg i.p.) 30 min before injection of isoprenaline, which reduced the resting heart rate from 418.1 ± 4 to 303.4 ± 8.9 beats/minute. Here, no apparent difference between the pempidine-treated and control animals was observed but, once again, a much greater sensitivity to intravenous isoprenaline was found in the pretreated animals, although the maximum tachycardia that could be produced after pretreatment was reduced.

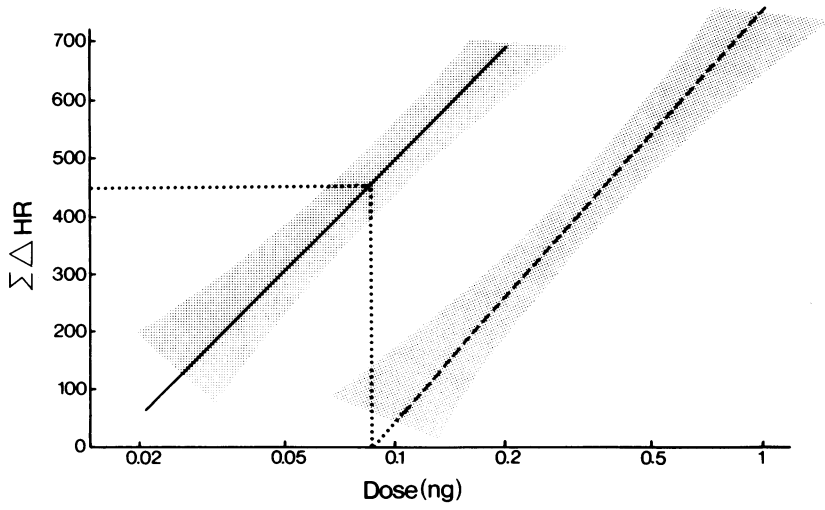

Figure 5 Dose-response relations for the effect upon heart rate of intravenously injected isoprenaline in reserpine-treated (unbroken line) and non-reserpine-treated mice (broken line), anaesthetized with pentobarbitone sodium and pretreated with atropine methylbromide. Each isoprenaline injection took approximately 2 s and the responses were measured over the subsequent 15 minutes. The shaded areas denote the 95% confidence limits. For reserpine-treated mice n = 53; for mice not treated with reserpine n = 50.

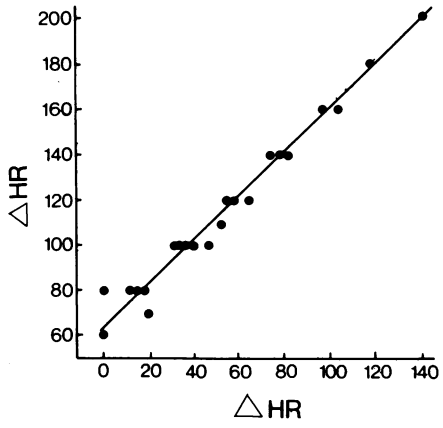

Figure 6 The relation between Δ HR values in groups of mice, measured at various intervals up to 15 min after the intravenous injection of isoprenaline following anaesthesia with pentobarbitone sodium and pretreatment with atropine methylbromide (abscissae), and corresponding values in mice additionally pretreated with reserpine, 3 mg/kg, 18 h earlier (ordinates). ($r = 0.986$, $n = 22$ pairs; $P < 0.001$).

Effect of pithing and adrenalectomy

The effectiveness of the doses of reserpine and pempidine in blocking the sympathetic nervous system was checked by using adrenalectomized mice and electrically stimulating the thoracic spinal outflow via the pithing rod. These experiments were carried out in atropinized mice, with and without pretreatment by reserpine or pempidine, and the maximum increase in heart rate produced by electrical stimulation for each animal was plotted against the voltage required to produce the effect. After pretreatment, an increase in heart rate beyond 30 beats/min could not be produced by stimulation with up to 100 V, whereas the minimum increase in control mice was 50 beats/minute.

Effect in spinal mice

Mice were spinalized as a further alternative means of blocking tachycardia of central origin. This did not affect the resting heart rate (from 397 ± 29 to 407 ±7.3 beats/minute). On injection of isoprenaline intracerebroventricularly, tachycardia developed only towards the end of the 15 min of observation (Figure 7). However, the response to intravenous isoprenaline in such mice was significantly greater than that in untreated mice

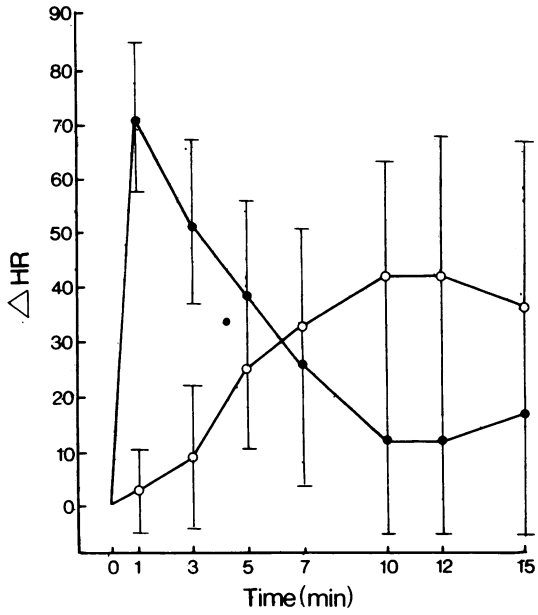

Figure 7 The effect upon heart rate of 10 ng isoprenaline injected intracerebroventricularly in groups of spinal (○) or intact mice (●), anaesthetized with pentobarbitone sodium and pretreated with atropine methylbromide. The vertical lines indicate s.e. mean.

(Figure 8), though less so than after reserpine- or pempidine-pretreatment.

Discussion

The poor responsiveness of mice pretreated with reserpine or pempidine to electrical stimulation of the thoracic cord outflow confirms that this pretreatment was adequate to block nervously-mediated tachycardia. The effect produced by intracerebroventricular isoprenaline in such mice must therefore be attributed to a direct action on the heart of amine leaking into the circulation.

The similarity between the effect of intracerebroventricular isoprenaline in untreated mice and that in mice pretreated with blocking agents suggests at first that this, too, is an effect consequent upon leakage. Two findings argue against this, however. It has been consistently observed that the increase in heart rate in reserpine-treated mice reaches a peak level later than in untreated mice. The values in untreated mice decline from 1 min after injection (Figure 3), suggesting that the two effects are not entirely identical.

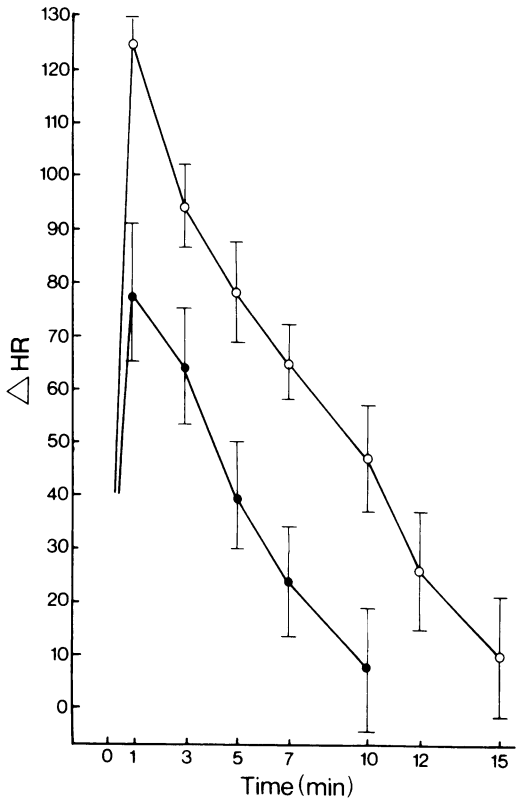

Figure 8 The effect upon heart rate of 0.5 ng isoprenaline injected intravenously in groups of spinal (○) or intact mice (●), anaesthetized with pentobarbitone sodium and pretreated with atropine methylbromide. The vertical lines indicate s.e. mean.

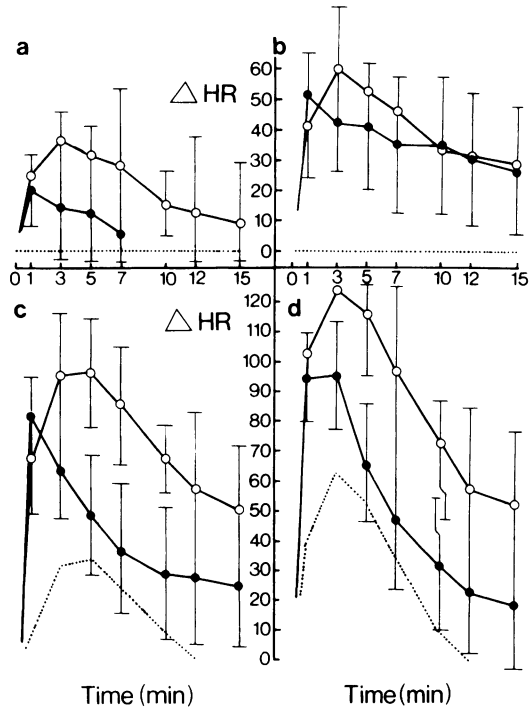

Figure 9 The effect upon the mean heart rate of groups of mice of doses of isoprenaline injected intracerebroventricularly after pretreatment with atropine methylbromide and anaesthesia with pentobarbitone sodium, with (○) or without (●) additional pretreatment with reserpine. The dotted line indicates the residual effect in reserpine-treated mice after correction for supersensitivity. Vertical lines indicate s.e. mean. Isoprenaline (a) 3.5 ng, (b) 5 ng, (c) 10 ng, (d) 20 ng. Section (a) is identical with Figure 3.

It has also been shown that pretreatment with reserpine results in supersensitivity to intravenous isoprenaline, such that the amine now appears five times as potent in causing tachycardia (Figure 5). A similar increase in response to isoprenaline was reported by Barrett & Carter (1970) in rats pretreated with syrosingopine. From Figure 5 it may be seen that any circulating isoprenaline causing an increase in heart rate of less than 450, expressed as $\Sigma\Delta$ HR, would not contribute to the increase seen in untreated mice.

The relationship derived in Figure 6 permits a correction to be applied to the values obtained for the tachycardia due to intracerebroventricular isoprenaline in reserpine-treated mice. In Figure 3 for example, it will be apparent that the degree of tachycardia seen after 5 ng isoprenaline in reserpine-treated mice would have been completely absent in untreated mice if the effects of isoprenaline were entirely peripheral. Thus, for this dose given intracerebroventricularly in such mice, the amount of amine leaking into the circulation is insufficient to contribute to the tachycardia observed in mice not treated with reserpine, which must therefore be of central origin. It is, however, an amount adequate to produce measurable tachycardia in supersensitive mice.

Figure 9 shows, for four doses of isoprenaline given intracerebroventricularly, the tachycardia after various intervals in atropinized mice and in mice additionally pretreated with reserpine, together with the residual values obtained by applying the above correction. It will be seen that only with doses above 5 ng is there any appreciable contribution from circulating amine;

reference to dose-response relations for intravenous isoprenaline suggests that, at peak, this represents no more than 10% of the injected dose. The effect represented by the difference between the dotted and lower unbroken lines in Figure 9 may thus be regarded as of central origin.

These conclusions are supported by the findings in spinal mice, in which intracerebroventricular isoprenaline caused only a slowly developing tachycardia, the initial peak effect being entirely absent (Figure 7). This effect must be attributed solely to a direct action of the amine consequent upon leakage, since all nervously mediated effect of central origin is precluded. It may be seen to resemble the effect remaining in reserpine-treated mice after applying the correction for supersensitivity (compare Figure 9c).

It appears, however, that some degree of supersensitivity is induced even by acute spinalization, since responses to intravenous isoprenaline were significantly greater in spinal mice than in controls (Figure 8). When allowance is made for this in a similar manner to that shown in Figure 6, the residual effects of intracerebroventricular doses of isoprenaline in spinal mice resemble closely those derived from reserpine-treated mice and shown in Figure 9.

Thus, the effects of intracerebroventricularly injected isoprenaline include tachycardia due both to a central action and a peripheral one consequent upon leakage of the amine into the systemic circulation. In this, the situation resembles that reported for noradrenaline by Cowell & Davey (1968) where, however, central and peripheral actions opposed each other whereas those of isoprenaline are similar. Gagnon & Melville (1967) and Bhargava *et al.* (1972) have shown that intracerebroventricular isoprenaline stimulated central β-adrenoceptors since β-adrenoceptor blocking agents injected by the same route blocked the effects. It may be presumed that the central components of the actions of isoprenaline in the mouse are similarly mediated, though leakage of β-adrenoceptor blocking agents after intracerebroventricular injection in mice would preclude a decisive experiment along these lines, since the circulating agent could affect a tachycardia of central origin by blocking peripherally.

The consequences of central β-adrenoceptor stimulation by isoprenaline include hypotension as well as tachycardia (Gagnon & Melville, 1967; Bhargava *et al.*, 1972). Reflex tachycardia evoked by the fall in blood pressure would be nervously mediated and indistinguishable from that directly due to central receptor stimulation by any of the methods used in the work reported here or by the authors quoted.

References

AHLQUIST, R.P. (1948). A study of the adrenotropic receptors. *Am. J. Physiol.*, **153**, 586-600.

BARRETT, A.M. & CARTER, J. (1970). Comparative chronotropic activity of β-adrenoceptive antagonists. *Br. J. Pharmac.*, **40**, 373-381.

BHARGAVA, K.P., MISHRA, N. & TANGRI, K.K. (1972). Analysis of central adrenoceptors for control of cardiovascular function. *Br. J. Pharmac.*, **45**, 596-602.

BRITTAIN, R.T. (1966). The intracerebral effects of noradrenaline and its modification by drugs in the mouse. *J. Pharm. Pharmac.*, **18**, 621-623.

COWELL, P. & DAVEY, M.J. (1968). The reversal of the central effects of noradrenaline by antidepressant drugs in mice. *Br. J. Pharmac.*, **34**, 159-168.

DAY, M.D. & ROACH, A.G. (1972). Adrenoceptors in the central nervous system concerned with cardiovascular control. *J. Pharm. Pharmac.*, **24**, Suppl. 148P.

GAGNON, D.J. & MELVILLE, K.I. (1967). Centrally mediated cardiovascular responses to isoprenaline. *Int. J. Neuropharmac.*, **6**, 245-251.

GILLESPIE, J.S. & MUIR, T.C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. *Br. J. Pharmac. Chemother.*, **30**, 78-87.

HALEY, T.J. & McCORMICK, W.G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Br. J. Pharmac. Chemother.*, **12**, 12-15.

KANEKO, Y., McCUBBIN, J.W. & PAGE, I.H. (1960). Mechanism by which serotonin, norepinephrine and reserpine cause central vasomotor inhibition. *Circulation Res.*, **8**, 1228-1234.

McCUBBIN, J.W., KANEKO, Y. & PAGE, I.H. (1960). Ability of serotonin and norepinephrine to mimic the central effects of reserpine on vasomotor activity. *Circulation Res.*, **8**, 849-858.

SHARE, N.N. & MELVILLE, K.I. (1964). Involvement of brain stem norepinephrine in picrotoxin and tyramine-induced central sympathetic stimulation. *Arch. int. Pharmacodyn.* **153**, 267-282.

SHARE, N.N. & MELVILLE, K.I. (1965). Intraventricular injections of picrotoxin following central adrenergic blockade with phenoxybenzamine and dichloroisoproterenol. *Int. J. Neuropharmac.*, **4**, 149-156.

SHAW, G.G. (1974). Some shortcomings of direct intraventricular injection in mice. *Br. J. Pharmac.*, **50**, 603-605.

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