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Modification of the vascular response to isoprenaline by cholinomimetic drugs

T. C. HAMILTON

Pharmacology Department, Roche Products Ltd., Welwyn Garden City, Herts

Summary

1. Pilocarpine and other cholinomimetic drugs convert isoprenaline to a vasoconstrictor and pressor agent.

2. This effect of pilocarpine was abolished by atropine ; it is thus an acetylcholine-like response. It was not dependent on the integrity of the central nervous system or the adrenal glands and was not abolished by ganglionic blockade.

3. The constrictor action of isoprenaline after pilocarpine was abolished by propranolol ; this action of isoprenaline is thus on the β-adrenoceptor. Another β-adrenoceptor stimulating agent, salbutamol, resembled isoprenaline in this situation, though papaverine and acetylcholine did not.

4. The constrictor action of isoprenaline after pilocarpine was abolished by phenoxybenzamine, guanethidine and cocaine ; the effect did not appear after reserpine pretreatment.

5. These results suggest an action of cholinomimetic drugs at adrenergic nerve endings which permits the uptake of β-adrenoceptor stimulating agents resulting in the release of neuronal transmitter.

Introduction

Fromherz (1946) first observed the reversal by pilocarpine of the response to isoprenaline from depressor to pressor in cats, and showed that this involved a cholinoceptor mechanism, since the depressor response to isoprenaline was restored by atropine. Daniell & Bagwell (1969) investigated this effect and found that pilocarpine no longer altered the hypotensive response to isoprenaline in dogs pretreated with reserpine. They concluded that noradrenaline, released from nerve endings by the ganglionic stimulant action of pilocarpine, occupied β-adrenoceptor sites thus preventing stimulation by the isoprenaline which, in this situation, then exerted an effect on α-adrenoceptors, leading to a pressor response.

Experiments in this laboratory, measuring vascular conductance in the autoperfused hindquarters and splanchnic region of the chloralosed cat, had indicated that the vasodilator effect of isoprenaline was not reversed to vasoconstriction after β-adrenoceptor blockade by propranolol, as also reported by Shanks (1967) for the perfused dog leg. This suggests that isoprenaline does not exert an effect on α-adrenoceptors as postulated by Daniell & Bagwell.

The work reported here was an attempt to elucidate the effect of pilocarpine and other cholinomimetic drugs upon responses to isoprenaline by studying their actions on the vessels of perfused vascular beds in the cat.

Methods

Cats of either sex between 1·8 and 3·0 kg body weight were anaesthetized with chloralose, 100 mg/kg intraperitoneally. Systemic blood pressure was recorded from a carotid artery (1 mmHg≡1·333 mbar) and the heart rate was monitored.

Vascular conductance in either the auto-perfused hindquarters or splanchnic region was recorded by the method described previously (Gardiner, Hamilton & Parkes, 1971). Essentially, this involves measurement of blood flow by an electromagnetic flow meter, using an extra-corporeal flow probe, in either the abdominal aorta or superior mesenteric artery, and of perfusion pressure distal to the flow probe. Electronic division of flow by pressure gives a continuous record of conductance changes simultaneously with those of blood flow and perfusion pressure.

Heparinized saline, 5 mg/kg, was injected intravenously immediately the cannulations were completed. Arterial injections were made via a side branch in the perfusion circuit, in a volume not greater than 0·2 ml, and washed into the bloodstream with 0·1 ml physiological saline. In some cats, reserpine, 3 mg/kg, was injected intraperitoneally in divided doses, 24 and 18 h before the experiment. Some other cats were prepared by pithing under ether anaesthesia.

Drugs used were acetylcholine iodide (B.D.H.), arecoline hydrobromide (Kodak), atropine methylbromide (McFarlane Smith), atropine sulphate (B.D.H.), cocaine hydrochloride (May & Baker), 1,1-dimethyl-4-phenyl piperazinium iodide (DMPP) (Emanuel), hexamethonium chloride (May & Baker), guanethidine sulphate (Ciba), DL-muscarine iodide (Prof. Eugster), nicotine hydrogen tartrate (B.D.H.), oxotremorine (May & Baker), papaverine hydrochloride (McFarlane Smith), pempidine tartrate (May & Baker), phenoxybenzamine hydrochloride (S.K.F.), phentolamine methanesulphonate (Ciba), pilocarpine hydrochloride (B.D.H.), practolol (I.C.I.), propranolol hydrochloride (I.C.I.), reserpine (Koch-Light), salbutamol sulphate (Allen & Hanburys), tyramine hydrochloride (B.D.H.).

Drugs were dissolved in saline and their doses are expressed as the salt except for oxotremorine and practolol, which are expressed as base. Reserpine was dissolved with the aid of acetic acid ; the dose is expressed as base. Isoprenaline (Burroughs Wellcome) and noradrenaline (Koch-Light) were prepared from stock ampoules of the sulphate and bitartrate, respectively, and their doses are expressed in terms of the base.

Results

Control responses

Intra-arterial injection of vasodilators in the hindquarters or splanchnic region of the cat produced a dose dependent increase of vascular conductance with a concomitant increase of blood flow and decrease of perfusion pressure, while vasoconstrictors produced the converse (Fig. 1).

With intravenous injection of vasodilators, the conductance of the hindquarters initially remained unaltered or fell transiently and this was followed by an increase (Fig. 2), while after noradrenaline the conductance initially increased briefly and then decreased (Gardiner, Hamilton & Parkes, 1971).

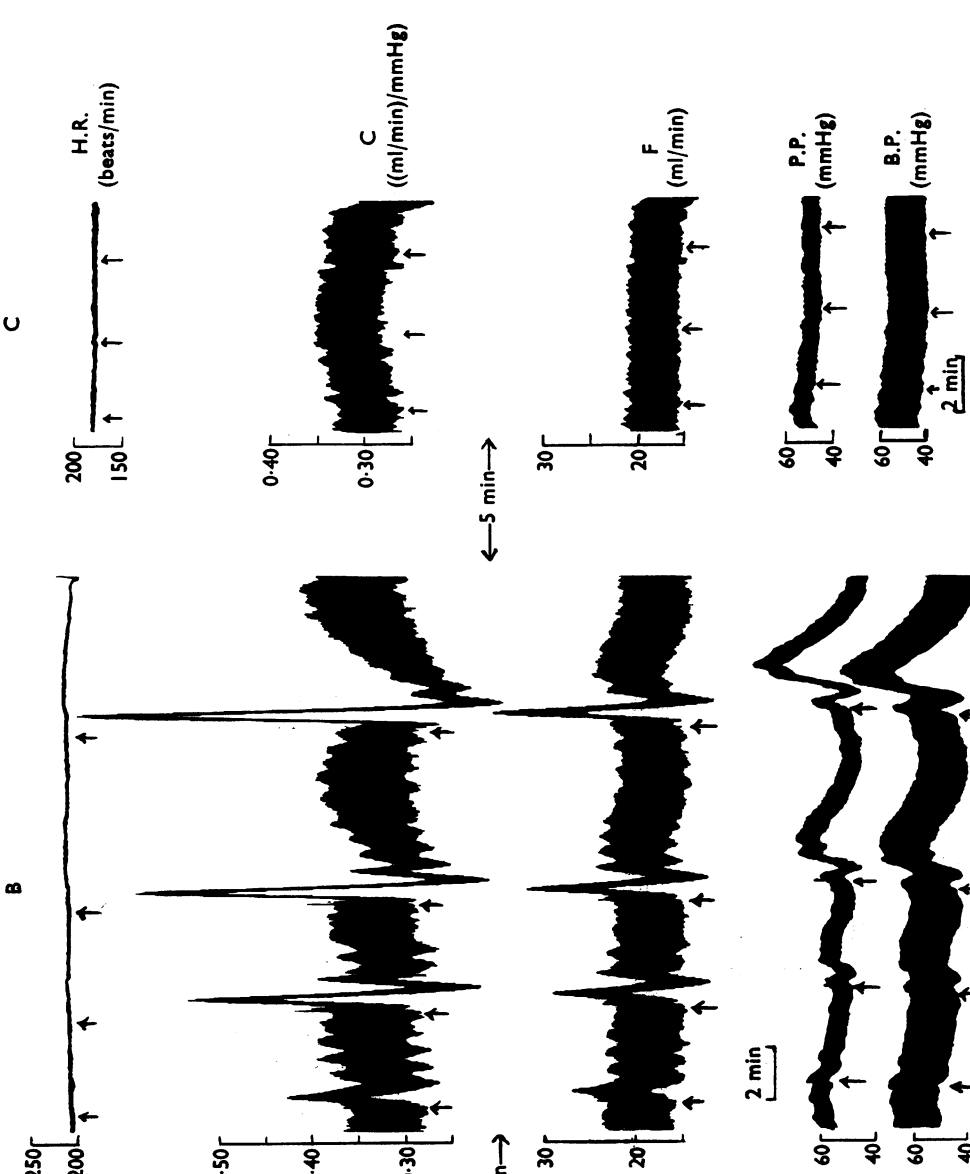

FIG. 1. Part of recording of hindquarters perfusion in a chloralosed cat. Traces from above downwards: heart rate (HR), vascular conductance (C), blood flow (F), perfusion pressure (PP), carotid blood pressure (BP). At each set of arrows, an injection of isoprenaline was made into the perfusion circuit: these show corresponding points on the traces and indicate the offset of the recording pens. The doses of isoprenaline were as follows: at 1, 0·02 μg; at 2, 0·05 μg; at 3, 0·10 μg; at 4, 0·20 μg. Between panels A and B, pilocarpine, 0·2 mg/kg, was injected and between panels B and C, propranolol, 0·1 mg/kg, both intravenously. Time intervals are shown on the record.

Effect of pilocarpine on isoprenaline responses

Pilocarpine reduced, or reversed to vasoconstriction, the dilator response to isoprenaline, 0·01–0·10 μg, injected into the perfused hindquarters or splanchnic region (Fig. 1); these responses were occasionally biphasic, dilatation being followed by constriction. In ten cats, the constrictor action was dose dependent, the percentage effect showing a relation to dose similar to that of the dilator action of isoprenaline before pilocarpine was given (Fig. 3; for dilation, $r=0{\cdot}596$; for constriction, $r=0{\cdot}505$; $n=25$). In eleven cats in which isoprenaline remained dilator after pilocarpine, the effect was still dose dependent, the shift in relation indicating antagonism.

After pilocarpine, 0·2 mg/kg intravenously, the depressor response to intravenous isoprenaline, 0·05–0·50 μg/kg, was reduced or abolished and a marked dose dependent pressor effect produced ($r=0{\cdot}905$, $n=14$), with a fall in conductance in the hindquarters (Fig. 2). The tachycardia due to isoprenaline was also markedly reduced or abolished. The threshold dose for these effects was 0·2 mg/kg pilocarpine intravenously or 0·1 mg pilocarpine injected into the perfusion circuit. The

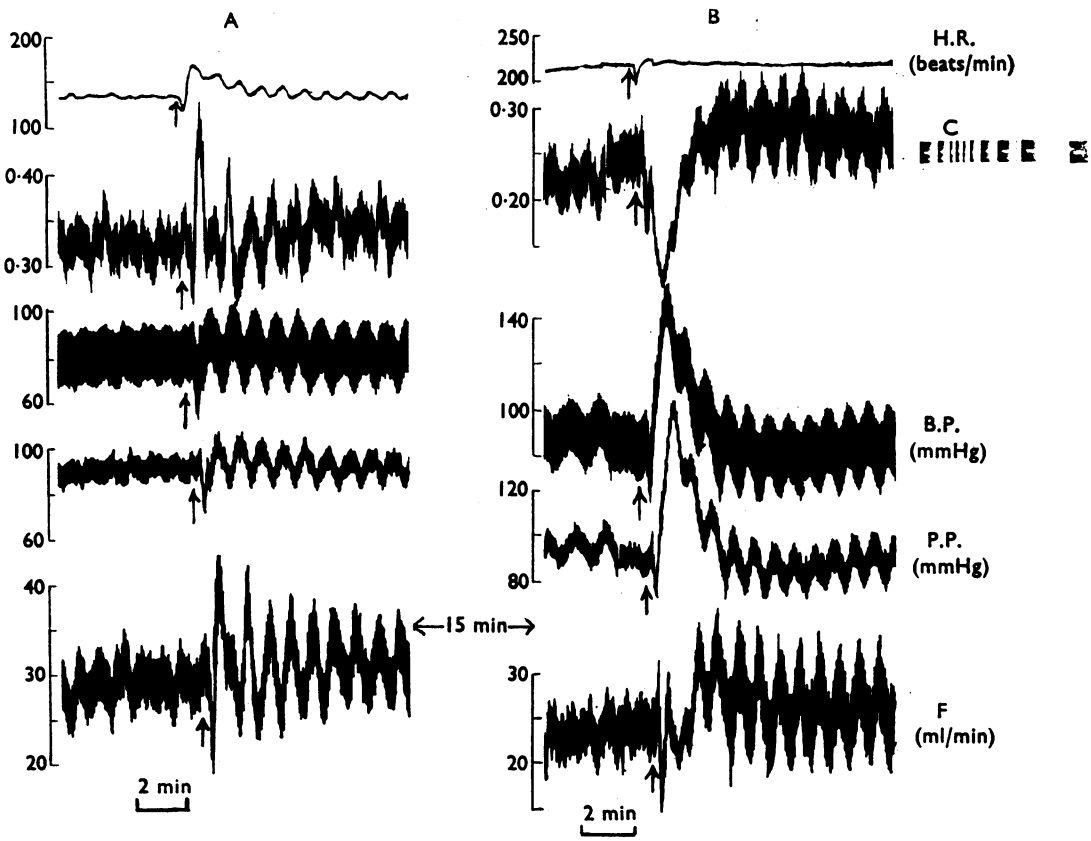

FIG. 2. Part of recording of hindquarters perfusion in a chloralosed cat. Traces, from above downwards: heart rate (HR), vascular conductance (C), carotid blood pressure (BP), perfusion pressure (PP), blood flow (F). At each set of arrows, an injection of 0·1 μg/kg isoprenaline was made intravenously: these show corresponding points on the traces and indicate the offset of the recording pens. Between panels A and B, pilocarpine, 0·2 mg/kg, was injected intravenously. Time intervals are shown on the record.

responses to isoprenaline returned to normal after 1 h but could be reversed again by a further dose of pilocarpine.

Atropine sulphate, 0·5 mg/kg, and atropine methylbromide, 0·5 mg/kg intravenously, reversed or prevented the action of pilocarpine on responses to isoprenaline. The effects of pilocarpine on isoprenaline responses persisted after pithing or after bilateral ligation of the adrenal glands. They were also observed in cats dosed intravenously with pempidine, 5 mg/kg, or hexamethonium, 10 mg/kg.

Propranolol, 0·1 mg/kg intravenously, abolished the pressor effect and fall in conductance with isoprenaline after pilocarpine (Fig. 1), while practolol, 1·0 mg/kg, had no effect. Phentolamine, 4 mg/kg intravenously, markedly reduced or abolished the pressor effect of isoprenaline and, in cats pretreated with 10–15 mg/kg phenoxybenzamine intravenously, no pressor response was observed, although some reduction of local dilator activity persisted.

Guanethidine, 2–4 mg/kg intravenously, prevented the pressor effect of isoprenaline, though pilocarpine still reduced dilatation ; constriction was not observed. Cocaine, 2–4 mg/kg intravenously, reversed or prevented the modification of isoprenaline responses by pilocarpine (Fig. 4). In cats pretreated with reserpine, pilocarpine did not alter responses to intravenous or intra-arterial isoprenaline. After section of the femoral and sciatic nerves high in each limb, pilocarpine continued to antagonize isoprenaline vasodilatation in the hindquarters.

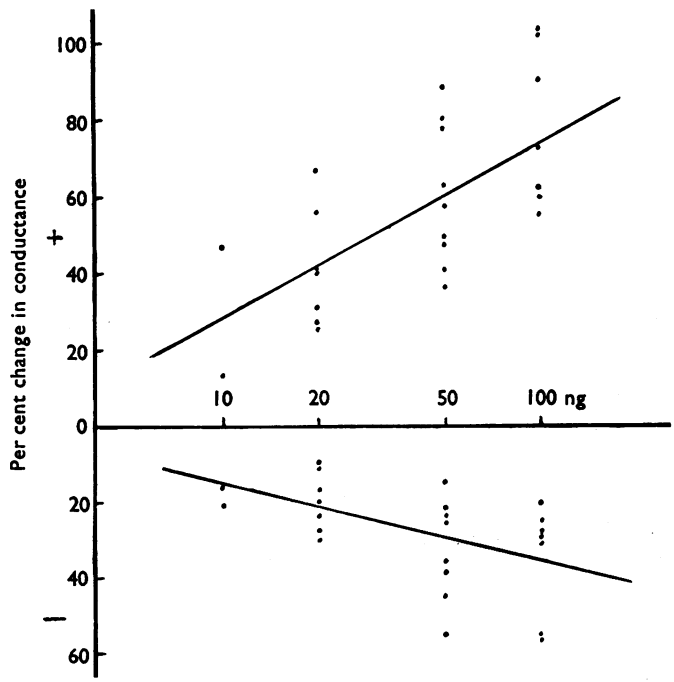

FIG. 3. Dose response relations for the effect of isoprenaline, injected into the perfusion circuit, upon vascular conductance of the chloralosed cat hindquarters before and after an injection of pilocarpine, 0·2 mg/kg intravenously, measured as percentage increase or decrease, respectively, of the predose level. Each line was calculated from twenty-five values (see text).

Effect of pilocarpine on responses to other vasoactive agents

Pilocarpine, 0·2 mg/kg intravenously, reduced or reversed the increase in conductance of the hindquarters produced by the intra-arterial injection of salbutamol, 0·05–0·5 μg, as with isoprenaline, while the depressor response to injection of salbutamol, 1·0 μg/kg, was converted to pressor. In contrast, pilocarpine did not significantly modify the vasodilator effects of papaverine (Fig. 5).

The pressor responses to intravenous doses of noradrenaline, 0·1–0·2 μg/kg, and tyramine, 0·1 mg/kg, were reduced after pilocarpine, but the depressor response to acetylcholine, 0·05–1·0 μg/kg intravenously, was unaffected.

Effect of other cholinomimetic agents on isoprenaline responses

Intravenous injection of oxotremorine, 2–10 μg/kg, arecoline, 0·2–0·5 mg/kg, or muscarine, 5–10 μg/kg, reduced or reversed the increase of conductance in the hindquarters produced by intra-arterial isoprenaline. In the presence of these cholino-

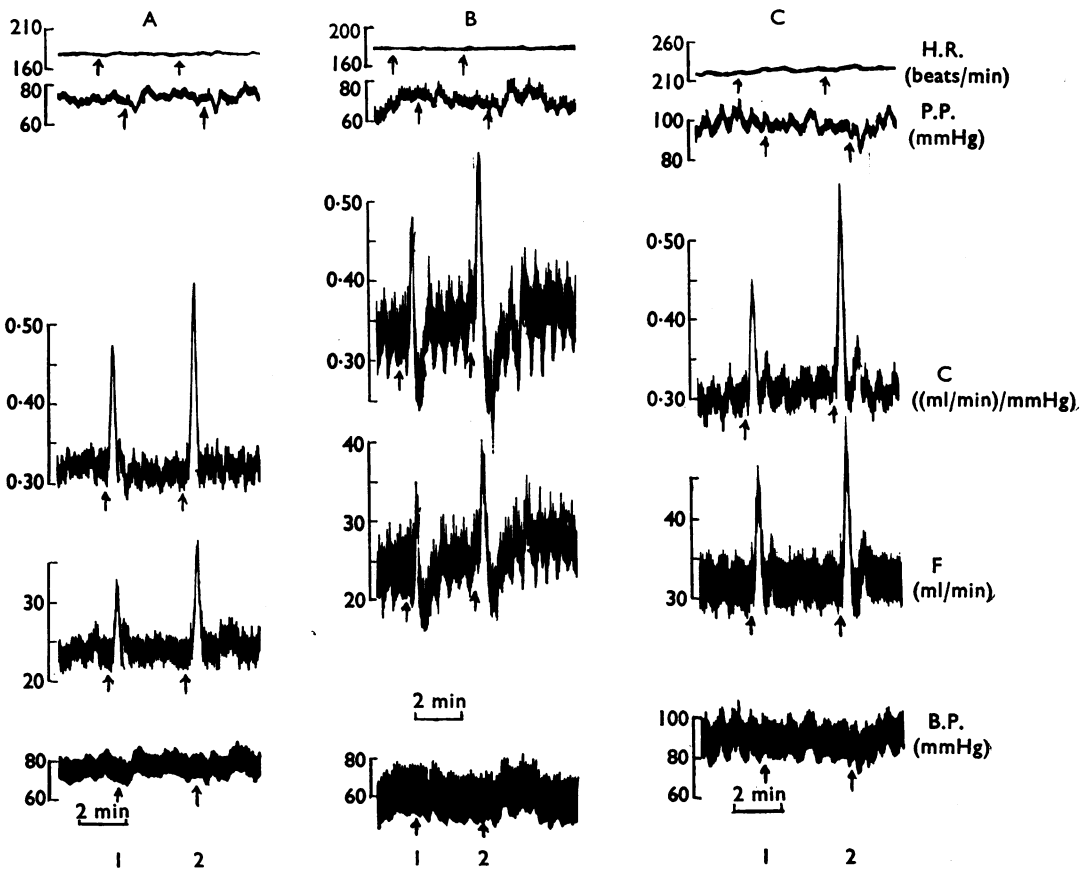

FIG. 4. Part of recording of hindquarters perfusion in a chloralosed cat. Traces, from above downwards: heart rate (HR), perfusion pressure (PP), vascular conductance (C), blood flow (F), carotid blood pressure (BP). At each set of arrows, an injection of isoprenaline was made into the perfusion circuit: these show corresponding points on the traces and indicate the offset of the recording pens. The doses of isoprenaline were as follows: at 1, 0·02 μg; at 2, 0·05 μg. Between panels A and B, pilocarpine, 0·2 mg/kg, was injected and between panels B and C, cocaine, 2 mg/kg, both intravenously. The time interval between panels A and B was 15 min and that between B and C was 10 minutes.

mimetic agents the depressor effect of isoprenaline and its tachycardia were reduced or abolished and a pressor response produced.

The effects of these drugs upon isoprenaline responses were reversed or prevented by cocaine, atropine methylbromide and guanethidine, as in the results described for pilocarpine. Propranolol also antagonized the pressor effects of isoprenaline after oxotremorine. In reserpinized cats, the cholinomimetic agents did not modify isoprenaline responses.

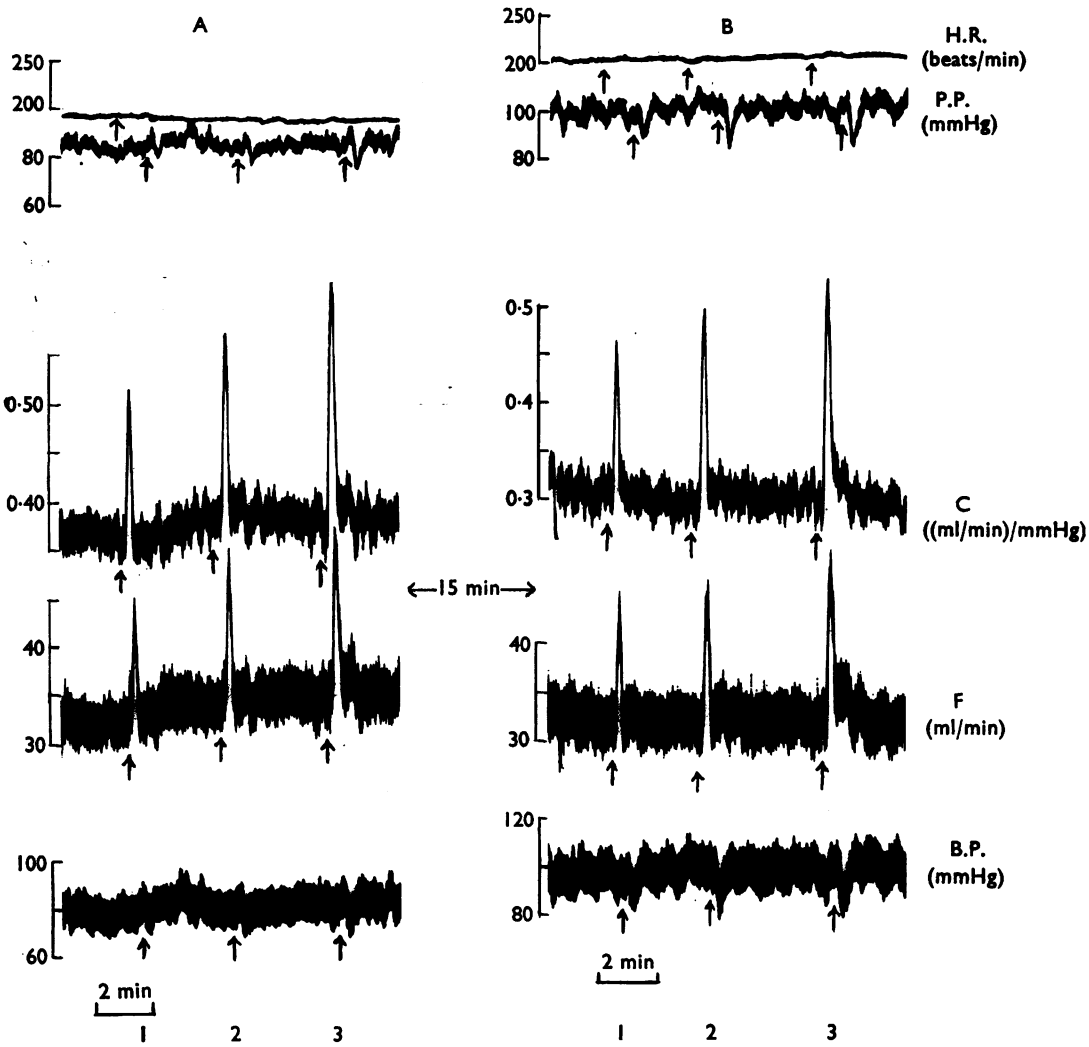

FIG. 5. Part of recording of hindquarters perfusion in a chloralosed cat. Traces, from above downwards: heart rate (HR), perfusion pressure (PP), vascular conductance (C), blood flow (F), carotid blood pressure (BP). At each set of arrows, an injection of papaverine was made into the perfusion circuit: these show corresponding points on the traces and indicate the offset of the recording pens. The doses of papaverine were as follows: at 1, 50 μg; at 2, 100 μg; at 3, 200 μg. Between panels A and B, pilocarpine, 0·2 mg/kg, was injected intravenously. Time intervals are shown on the record.

Effect of nicotine and DMPP on isoprenaline responses

Neither nicotine, 0·5–1·0 mg/kg, nor DMPP, 10–100 μg/kg intravenously, affected the depressor action or tachycardia due to intravenous isoprenaline.

Discussion

The abolition by atropine of the effect of pilocarpine in converting isoprenaline to a vasoconstrictor agent, demonstrates the acetylcholine-like nature of the effect, as previously reported by Fromherz (1946) and Daniell & Bagwell (1969). This is further supported by the finding that other cholinomimetic agents such as muscarine, arecoline and oxotremorine behaved as did pilocarpine. The effect is unlikely to be exerted centrally, since atropine methyl bromide was as effective as atropine in blocking this action of pilocarpine, while the action persisted after pithing. Nor is it likely to involve ganglionic stimulation by pilocarpine, as suggested by Daniell & Bagwell (1969), since neither hexamethonium nor pempidine blocked the effect. Moreover, the ganglionic stimulants nicotine and DMPP did not modify responses to isoprenaline, whereas oxotremorine, which lacks ganglionic stimulant action (Cho, Haslett & Jenden, 1962) resembled pilocarpine in its effect on isoprenaline.

The vasoconstrictor action of isoprenaline after pilocarpine was shown to be caused by stimulation of the β-adrenoceptor because it was abolished by propranolol. There is no evidence for partial α-adrenoceptor stimulation by isoprenaline, as suggested by Daniell & Bagwell (1969), since vasodilatation by this amine was not potentiated by α-adrenoceptor blockade; were there such a stimulus, this could limit the degree of dilatation appearing in the absence of such a blockade.

Nevertheless, α-adrenoceptor blocking agents could prevent vasoconstriction by isoprenaline after pilocarpine, allowing vasodilatation to reappear. Further, in cats pretreated with reserpine, isoprenaline was only vasodilator in the presence of pilocarpine, in agreement with the findings of Daniell & Bagwell (1969). This evidence suggests mediation of the response by endogenous noradrenaline and this is also supported by the prevention of the effect by guanethidine. This further implies that release of transmitter from adrenergic neurones is involved, which is substantiated by the action of cocaine. In doses that potentiate the pressor actions of injected noradrenaline and reduce those of injected tyramine, cocaine prevented or reversed the modification of the isoprenaline response by pilocarpine and other cholinomimetic agents.

The effect of pilocarpine appeared to be specific for β-adrenoceptor stimulating agents since among vasodilator drugs it affected salbutamol in addition to isoprenaline but not papaverine or acetylcholine. Further, since the depressor action of acetylcholine and the pressor action of noradrenaline were not potentiated after pilocarpine, an increase in the sensitivity of muscarinic or α-adrenoceptors is not involved.

These results suggest that in the presence of cholinomimetic agents, isoprenaline is taken up into adrenergic nerve endings and stimulates the release of noradrenaline. The uptake mechanism is sensitive to blockade by cocaine and propranolol while reserpine depletes the transmitter pool susceptible to release by isoprenaline. As a result of its uptake, less isoprenaline may be available to combine with peripheral β-adrenoceptors, leading to reduced dilatation of the blood vessels, which are constricted by the released noradrenaline.

Ross (1963) and Hertting (1964) showed that isoprenaline was taken up to only a slight extent by the tissues of mice and rats *in vivo*. Iversen (1967) reported that isoprenaline was not taken up by the Uptake$_1$ process in isolated rat heart, though it was removed by Uptake$_2$, a process which is less sensitive to inhibition by cocaine than is Uptake$_1$; Davidson & Innes (1970), however, showed that isoprenaline uptake in cat spleen must differ from that shown by Iversen for rat heart. The cholinomimetic drugs may act at adrenergic neurones to promote the uptake of isoprenaline in preference to noradrenaline ; Rand & Varma (1970) have reported that pilocarpine facilitated release and impaired uptake of noradrenaline at adrenergic nerve endings.

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