SOME PHARMACOLOGICAL PROPERTIES OF A COMMERCIAL HEART EXTRACT

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Received March 11, 1959

An investigation was made of the pharmacological properties of a commercial heart extract (Recosen), said to be of value in the treatment of angina pectoris. This extract increased coronary flow but decreased force of beat in isolated perfused mammalian hearts. It potentiated the pressor effects of adrenaline in the spinal cat whilst not affecting those of noradrenaline, and it antagonised the actions of adrenaline and noradrenaline upon intestinal muscle. A mild anticholinesterase activity was demonstrated on striped muscle. The significance of these findings is discussed.

The work described in this paper was performed in an attempt to clarify the pharmacological properties of Recosen, a protein-free water-soluble preparation made from the hearts of freshly killed healthy young pigs, horses, and sheep. This extract is said to increase the coronary blood flow in the hearts of dogs[1], guinea pigs[2], and cats[3], and to increase the mechanical efficiency of the dog's heart by depressing cardiac metabolism[4]. It has been used clinically in the treatment of angina pectoris, and favourable results have been reported from its use in this disease[5,6]. It is also claimed to reduce the toxicity of digitalis alkaloids[7].

In spite of the fact that no specific active principle has as yet been isolated from this extract, it was felt that any substance which was claimed to be of value in the treatment of angina pectoris was worthy of investigation. Experiments were therefore performed which might determine or confirm the cardiac and other general pharmacological actions of this extract. Attempts were also made to identify by biological methods a specific active constituent of this mixture.

Methods

Spinal cats. Cats were spinalised either under intraperitoneal pentobarbitone (30 mg./kg.) or ether anaesthesia. Blood pressure was recorded from a carotid artery by a mercury manometer, and injections of drugs were given into an external jugular vein.

Intestine. Rabbit duodenum was suspended in Locke's solution at 37° through which was bubbled a mixture of 95 per cent O_2 and 5 per cent CO_2. Contractions were recorded with a frontal writing lever on a kymograph.

Perfused hearts. Isolated mammalian hearts were perfused with Locke's solution at 37° using a modified Langendorff apparatus[8]. The constitution of the Locke's solution was NaCl, 9·0, KCl, 0·42, $CaCl_2$, 0·24, dextrose, 1·0, $NaHCO_3$, 0·5 g., distilled water to 1000 ml., and it was

oxygenated with a mixture of 97 per cent O_2 and 3 per cent CO_2. Coronary flow was recorded on the input side of the apparatus using either a photo-transistor or a platinum wire drop recorder, feeding a Thorp impulse counter. Drugs were given either by the injection of single doses into the coronary inflow, or by continuous perfusion of fixed concentrations.

Frog rectus muscle. Frog rectus abdominis muscle was suspended in Ringer's solution and aerated at room temperature. Contractures were recorded on a kymograph by a gimbal lever.

RESULTS

Physical Properties

The Recosen used in these experiments was supplied in ampoules, each ampoule containing "heart extract", 0·014 ml., *m*-cresol, 0·003 ml., distilled water to 1 ml. All doses of heart extract stated in this paper refer to amounts of the commercially available ampoules, and not to the pure heart extract. These ampoules contained a brown odourless fluid, pH 6·5, which exhibited a marked fluorescence under ultra-violet light. Full activity was retained after boiling for 3 hours, and after prolonged acid or alkaline hydrolysis.

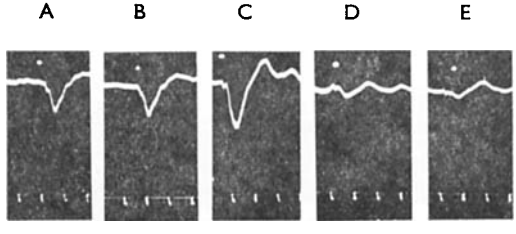

FIG. 1. Spinal cat, 2·7 kg. Upper tracings show blood pressure and lower tracing is time marker (30 sec.). At A and B, 0·5 ml. of heart extract injected. Between A and B, atropine sulphate, 0·5 mg./kg. At C, histamine, 3 μg. Between C and D, mepyramine maleate, 1 mg./kg. At D, histamine, and at E, heart extract, both in the same doses as before.

Spinal Cats

Cardiovascular responses to the heart extract were studied in seven cats. When this extract was given intravenously in doses of 0·1–2·0 ml./kg. there was a fall in blood pressure resembling that caused by histamine. This effect was not affected by the prior administration of atropine, but could be totally abolished by suitable doses of the antihistamines, diphenhydramine and mepyramine (Fig. 1).

Quantitative assay by this blood pressure effect revealed a histamine content of 3 μg./ml. of heart extract. This would be in accord with the results communicated to me by Kocher, who estimated the histamine content of the extract as 2–3 μg./ml. That this effect was not due to histamine release by the extract was shown by the fact that the fall in blood

pressure was immediate in onset, and there was no initial delay as is seen with histamine-releasing compounds.

The most remarkable effect of this extract seen in the spinal cat was a differential potentiation of the pressor actions of adrenaline and noradrenaline. Doses of 0·5–2·0 ml./kg. of heart extract increased the hypertensive response to adrenaline whilst having a negligible effect on the pressor action of noradrenaline.

The potentiated pressor response to adrenaline was accompanied by an increased tachycardia; there was no alteration in the effect of noradrenaline on heart rate. This action of the extract was markedly different from the potentiation of adrenaline and noradrenaline caused by cocaine, in which the pressor effects of the two amines were potentiated to about the same degree (Fig. 2), and were accompanied by a pronounced increase in the response of heart rate to noradrenaline with only a slight increase in this response to adrenaline.

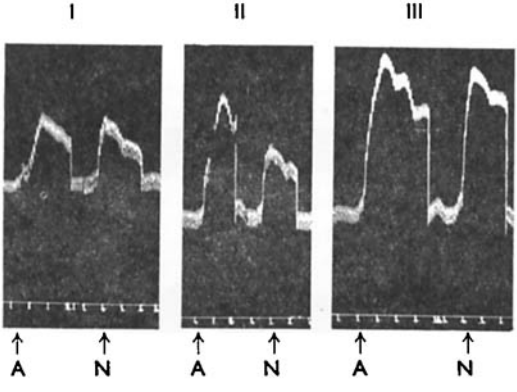

FIG. 2. Spinal cat, 3·9 kg., showing the effects on blood pressure of injection of 10 μg. adrenaline (A) and 1 μg. noradrenaline (N). I is a control; II is after 2 ml. of heart extract; III is after 10 mg. cocaine intramuscularly.

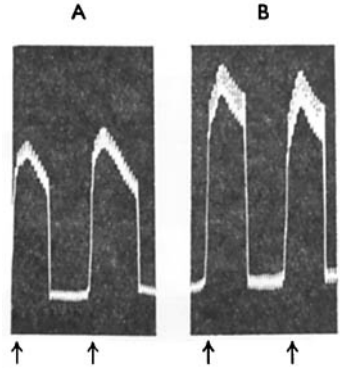

FIG. 3. Spinal cat, 3 kg. Tracing shows blood pressure effects of intravenous injection of 10 μg. adrenaline (indicated by arrows). A is before and B is after the injection of 1·5 ml./kg. of heart extract.

This potentiating action of single doses of the heart extract was prolonged in duration, giving the same quantitative results after 4 hours as it did after 10 minutes.

Differential potentiation of the pressor actions of adrenaline and noradrenaline has not been previously described for this extract. It seemed possible that the mechanism underlying this potentiation could be either an increase in the excitatory cardiovascular actions of adrenaline, or a decrease in the peripheral vasodilator response to adrenaline. Further experiments were therefore made to discover whether either or both of these phenomena could be demonstrated.

Rabbit Duodenum

As adrenaline has an inhibitory effect on the smooth muscle of the gastrointestinal tract similar to its action on the smooth muscle of certain

peripheral vascular beds, eight experiments were performed on rabbit duodenum to see whether the action of adrenaline on this preparation was modified in any way by the heart extract. In these experiments concentrations of the extract of 0·1–1·0 ml. per litre had no action of their own upon intestinal tone or spontaneous contractions, but these concentrations of the extract greatly reduced the inhibition of tone and motility caused by adrenaline. This effect was more pronounced with noradrenaline than with adrenaline. Thus, in the presence of 1·0 ml. of heart extract per litre, the inhibition of tone due to adrenaline was reduced by 32·5 per cent (S.D $\pm$ 1·5 per cent) whilst the inhibition caused by noradrenaline was reduced by 58 per cent (S.D. $\pm$ 4 per cent).

Isolated Mammalian Hearts

The actions of the heart extract were studied upon rabbit, guinea pig, and kitten hearts, to confirm results previously reported claiming a coronary vasodilator action of this extract[1–3], to assess its actions upon force of beat, and to study any modification that it might produce upon the actions of sympathomimetic amines on isolated hearts.

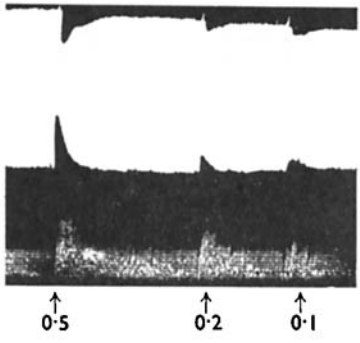

FIG. 4. Isolated perfused rabbit heart, showing responses to 0·5, 0·2 and 0·1 ml. of heart extract. Upper tracing shows myocardial contractions, lower tracing shows coronary flow, the height of each vertical line indicating flow in drops per five seconds.

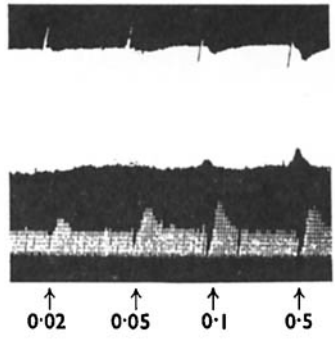

FIG. 5. Isolated perfused guinea pig heart, showing responses to 0·02, 0·05, 0·1 and 0·5 ml. of heart extract. Upper and lower tracings are as for Figure 4.

When single doses of the extract were given, a pronounced species difference was noted between the effects on rabbit hearts and those on the other species studied. In 10 experiments on rabbit hearts, doses of up to 0·1 ml. were usually without effect. This lack of effect was not due to lack of reactivity of the heart, as vasodilatation of the coronary vessels could still be produced with glyceryl trinitrate, and vasoconstriction with adrenaline. Doses of 0·1–0·5 ml. of the extract increased coronary flow, but also caused marked decrease in the force of the heart beat (Fig. 4). Doses of more than 0·5 ml. usually caused transient cardiac arrest, and the beat, when resumed, remained of reduced amplitude.

In 11 experiments on guinea pig hearts, single doses of the extract had a much greater effect on coronary flow than on the force of beat (Fig. 5). Doses of 0·001–0·05 ml. caused a marked increase in the coronary flow without affecting the force of beat. Higher doses caused similar, though much less marked, actions to those seen with rabbit hearts. In neither species were there any consistent alterations in heart rate when the heart extract was given in non-toxic doses. In 3 kitten hearts studied, the effects on coronary flow and force of beat were similar to, though less pronounced than, those seen in the guinea pig heart, but in this species these effects were accompanied by a moderate increase in the heart rate.

In view of the alterations in response to adrenaline and noradrenaline caused by the extract in spinal cats, attempts were made to see whether the heart extract modified in any way the coronary vasodilatation that these amines cause in kitten hearts, and the coronary vasoconstriction that they produce in isolated perfused rabbit and guinea pig hearts. But, when continuous perfusions of Locke's solution containing 0·001–0·1 ml. of extract per litre were used, it was not possible to demonstrate any alteration in the cardiac actions of adrenaline or noradrenaline. Concentrations of more than 0·1 ml./litre caused cardiac irregularities which obscured the effects of the sympathomimetic amines.

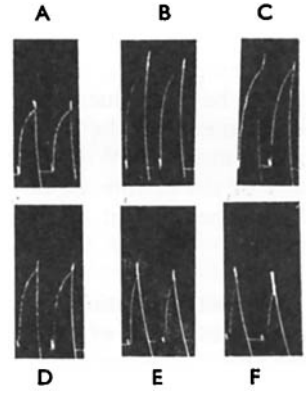

FIG. 6. Frog rectus abdominis muscle, showing contractures produced by A, Acetylcholine (ACh), 0·5 μg./ml. B, ACh, 1·0 μg./ml. C, ACh, 0·5 μg./ml. + heart extract, 0·2 ml./ml. D, ACh, 0·5 μg./ml. + heart extract, 0·1 ml./ml. E and F show contractures in the presence of ethyl pyrophosphate, 5 μg./ml. E, ACh, 0·1 μg./ml. F, ACh, 0·1 μg./ml. + heart extract, 0·2 ml./ml.

Striped Muscle

In view of the deleterious effect that larger doses of the heart extract had on cardiac muscle of certain species, a series of experiments were performed on frog rectus muscle, a simple striped muscle preparation, to see whether similar deleterious effects could be reproduced in skeletal muscle. In 6 experiments on this preparation, the heart extract in doses of 0·02–0·2 ml./ml. of Ringer's solution had no direct action on the muscle, but potentiated contractures caused by acetylcholine. Carbachol-induced contractures were unaffected, and the potentiation of acetylcholine-induced contractures was abolished after treatment with the anticholinesterases, physostigmine and ethyl pyrophosphate (Fig. 6).

EXPERIMENTS ON THE CHEMICAL CONSTITUENTS OF THE EXTRACT

(*a*) In view of the marked fluorescent properties of the heart extract, solutions of flavine adenine dinucleotide were prepared, ranging in concentration from 0·001 μg./ml. to 10 mg./ml., since this compound is a

riboflavine precursor which exhibits marked fluorescence and which has been isolated from pig heart[9].

(*b*) A number of amines and amino acids have been determined in the heart extract (Kocher, personal communication), and its constitution has provisionally been stated as follows (concentrations are given in μg./ml.):— histamine, 3; acetylcholine, less than 0·1; catechol derivatives, 0; adenosine and derivatives, 2; arginine, 130–150; cystine, 90–120; histidine, 60–70; leucine, 160–190; methionine, 40; tryptophane, 18; tyrosine, 60; potassium, 510; sodium, 340; magnesium, 95; calcium, 40. A solution of this constitution was prepared, using the laevorotatory forms of the amino acids and the upper limits of the concentrations stated. Adenosine triphosphate was used in place of the "adenosine and derivatives" stated above.

Attempts were made to see whether the effects of the heart extract could be reproduced by solutions (*a*) and (*b*) when they were used in experiments on the spinal cat, perfused hearts, rabbit duodenum, and frog rectus muscle. With neither of these solutions was it possible to reproduce any of the actions of the heart extract, apart from the effects due to the histamine content of solution (*b*) upon the blood pressure of the spinal cat.

Discussion

A most interesting result of the above experiments was the modification of the responses of the spinal cat to adrenaline and noradrenaline, where the effects of adrenaline on blood pressure and heart rate were potentiated but those of noradrenaline were unaffected. It has been shown by Innes[10] that the potentiation of sympathomimetic amines caused by cocaine and by certain antihistamines is accompanied by a rise in the heart rate response to noradrenaline with no alteration in this response to adrenaline. The heart extract appeared to produce the opposite phenomenon to this.

The increased pressor response to adrenaline after injections of this extract may be caused by an increase in the excitatory cardiovascular actions of adrenaline (i.e., an increased cardiac output or increased vasoconstriction), but it may also be due to a modification of the peripheral inhibitory actions of adrenaline. The pressor response to adrenaline is normally accompanied by vasodilatation in certain peripheral vascular beds, such as those in the liver and in skeletal muscle. It is possible that the heart extract antagonises this peripheral vasodilator effect of adrenaline, increasing the total peripheral resistance and so causing an increased rise in blood pressure.

This suggested mode of action of the heart extract in potentiating the pressor response to adrenaline by altering the peripheral response to this drug is supported in part by the actions seen in the rabbit duodenum. In this preparation sympathomimetic amines usually cause a relaxation of tone and an inhibition of spontaneous contractions, a manifestation of the properties of these amines in relaxing smooth muscle. This effect was greatly reduced in the presence of the heart extract, and it is possible that similar effects may occur on the smooth muscle of peripheral blood vessels. This hypothesis is not invalidated by the observation that in the

rabbit duodenum the heart extract had a greater inhibitory effect on the responses to noradrenaline than it did on those to adrenaline. The pressor effect of noradrenaline, unlike that of adrenaline, is not accompanied by significant peripheral vasodilatation. If the heart extract did potentiate the pressor effect of adrenaline by inhibiting the peripheral vasodilatation caused by sympathomimetic amines, it would not by this action affect the pressor response to noradrenaline.

The results elicited in isolated mammalian hearts confirm the results of Haemmerli[11], and of Ryser and Wilbrandt[2], who showed that this extract caused a dilatation of the coronary arteries in guinea pig hearts. Increased force of heart beat was not seen with the heart extract in these experiments; high doses of the extract decreased the force of beat in all species examined, an effect that was much more marked in rabbit hearts than in those of the other species.

The results of the experiments on frog rectus muscle, in which the extract potentiated acetylcholine contractures whilst not affecting those produced by carbachol, and in which this potentiation was abolished by the addition of anticholinesterases, suggest that the heart extract has a mild anticholinesterase activity.

The investigations performed on the constituents of the extract show that its action cannot be attributed to any of the substances so far identified in it. If the fluorescent property of the extract is due to the presence of flavine adenine dinucleotide, this fraction would also appear to be devoid of biological activity. Ryser and Willbrandt[2] in experiments on the coronary resistance of the isolated guinea pig heart demonstrated that this extract had a different dose-response curve to that of adenylic acid and other adenosine derivatives. Whilst it is still possible that the actions of the extract are due to the presence in it of an adenosine derivative, the actions of which are modified by some other constituent, the stability of the extract, and the failure of solutions of adenosine triphosphate to cause similar actions to those of the extract make this possibility unlikely.

Since it is not possible to draw comparisons between the results of acute laboratory experiments performed on healthy experimental animals and the therapeutic results of trials of the drug on patients who suffer from chronic cardiac disorders and who are on a prolonged course of therapy, the results of the experiments described in this paper should not be construed as detracting from the therapeutic properties claimed for this extract. True assessment of the therapeutic worth of this drug will not be possible until there is a careful clinical trial of the double-blind type. It is understood that such a trial is at present in progress. The effects of this drug in dilating the coronary vessels, and its relationship to the actions of sympathomimetic amines would seem to be worthy of further pharmacological study.

Acknowledgements. I am greatly indebted to Dr. J. B. E. Baker for much helpful advice on the planning of these experiments and in the preparation of this paper. The Recosen used in these experiments was made available by Dr. N. Levinson, of Robopharm Ltd., to whom I am also indebted for supplying much useful information about this drug.

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