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Studies on the action of acetylcholine on the sino-auricular node of the dog

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1. The response of the sino-auricular node to direct injection of acetylcholine in the artery supplying the node has been studied in the dog.

2. Acetylcholine in graded doses (2, 5, 10 μg) caused acceleration of the sinus rhythm.

3. The time course of the positive chronotropic action of acetylcholine resembled that produced by adrenaline or noradrenaline.

4. The positive chronotropic action produced by acetylcholine was blocked by dichloro-isopropylnoradrenaline and pronethalol and potentiated by cocaine.

5. In reserpinized dogs, acetylcholine in similar doses produced sinus bradycardia. The positive chronotropic response could be temporarily restored by infusion of adrenaline or noradrenaline.

6. Atropine blocked both the positive and negative chronotropic actions of acetylcholine.

The mechanism of the inhibitory action of acetylcholine on the heart is well established. In certain experimental conditions cardio-acceleration by acetylcholine has also been reported (McDowall, 1945). It is possible that acetylcholine activates the adrenergic mechanism of the sino-auricular (S.A.) node as it does at some other adrenergic sites (Burn & Rand, 1962). It has in fact been shown that acetylcholine may release an adrenaline-like substance from the perfused heart (Hoffman, Hoffman, Middleton & Yalesnik, 1945).

In this work the effect of acetylcholine was studied after direct injection into the artery supplying the S.A. node (intranodal injection) in order to avoid concurrent effects on other excitable or contractile tissues of the heart. The effects were compared with those produced by catecholamines, and attempts were made to analyse the mechanisms involved.

Methods

Mongrel dogs of either sex weighing 10–18 kg were anaesthetized with pentobarbitone sodium (30 mg/kg, intravenously) and supplementary doses were given as required. The trachea was intubated and respiration was maintained by positive pressure artificial respiration.

The chest was opened with a midsternal incision, and the pericardium opened to form a cradle for the heart. The right coronary artery was dissected out, freed from the surrounding fat, and cannulated with a polyethylene tube (internal diameter 0·23 inch, outer diameter 0·38 inch). The tip of the polyethylene tube was gently pushed into the artery until it reached the origin of the sinus node artery. The rest of the procedure was basically the same as described by James & Nadeau (1962). The cannula was then connected to a three-way stopcock, and flushed occasionally with a 0·5% solution of heparin in normal saline. All injections were made through the three-way stopcock. James & Nadeau (1963) reported that direct injection into the nodal artery leads to bradycardia in 90% of cases due to mechanical stretching. Stretching of the nodal region has also been reported to cause cardio-acceleration (Brooks, Lu, Lange, Mangi, Shaw & Geoly, 1966). In all experiments, injections were given with uniform pressure in a constant volume of 1·0 ml. during 30 sec.

Drugs used were: acetylcholine chloride (E. Merck); adrenaline and noradrenaline were prepared from the bases and ascorbic acid was added to the solution (pH 3·6); dichloro-isopropylnoradrenaline hydrochloride (Lilly); atropine sulphate; cocaine hydrochloride (E. Merck); pronethalol hydrochloride (I.C.I.). Reserpine (Serpasil, Ciba) was injected in a dose of 0·5 mg/kg, 48 and 24 hr before the experiment. In some reserpinized animals intranodal infusion of 60 μg of adrenaline or noradrenaline was given in 3 ml. of saline during a period of 3 min. The doses given in the text are total doses, and refer to the salts.

The electrocardiograph (lead II) was recorded continuously. Arterial blood pressure was recorded from a cannula in the left common carotid artery using a mercury manometer.

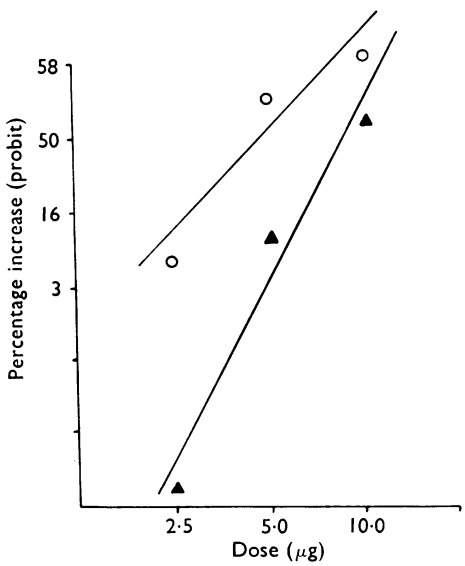

FIG. 1. Percentage increase in heart rate produced by the intranodal injection of graded doses of acetylcholine in the dog. (Results from two experiments.)

Results

Effect of acetylcholine on the S.A. node

Acetylcholine injected intranodally in doses of 2, 5 and 10 μg produced cardio-acceleration. The percentage increase in the heart rate in two typical experiments has been plotted as probits against dose in Fig. 1. Seven experiments were performed.

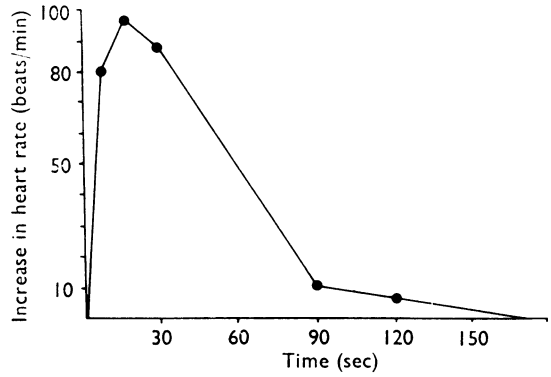

FIG. 2. Increase in heart rate (beats/min) over resting values after the intranodal injection of 5 μg of acetylcholine in the dog plotted against time after injection.

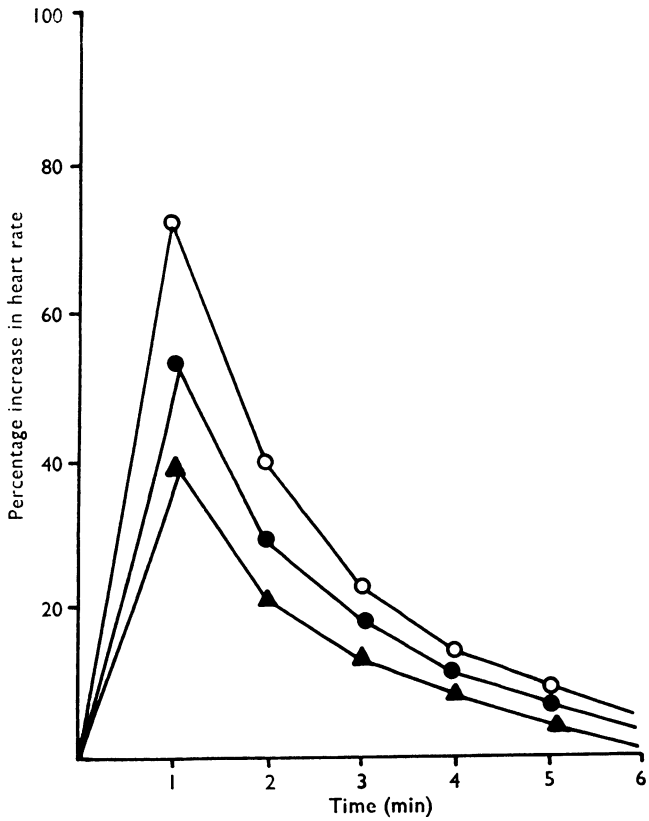

FIG. 3. Percentage increase in heart rate produced by intranodal injections in the dog of 5 μg each of noradrenaline (○——○), acetylcholine (●——●) and adrenaline (▲——▲), plotted against time after injection.

Maximum increase in the heart rate occurred 15–20 sec after the injection of acetylcholine, and this returned to normal within 3 min (Fig. 2). Acetylcholine given intravenously in similar doses caused typical bradycardia in the majority of dogs. Intranodal injections of 15–25 μg of acetylcholine produced bradycardia and doses of 1 μg were ineffective.

Effect of adrenaline and noradrenaline

In eight experiments both adrenaline and noradrenaline administered intranodally in doses ranging from 2 to 10 μg produced positive chronotropic responses in the heart. The speed of onset and duration of the responses of the S.A. node to acetylcholine, adrenaline and noradrenaline were similar, but noradrenaline was the most potent, followed by acetylcholine and then by adrenaline (Fig. 3).

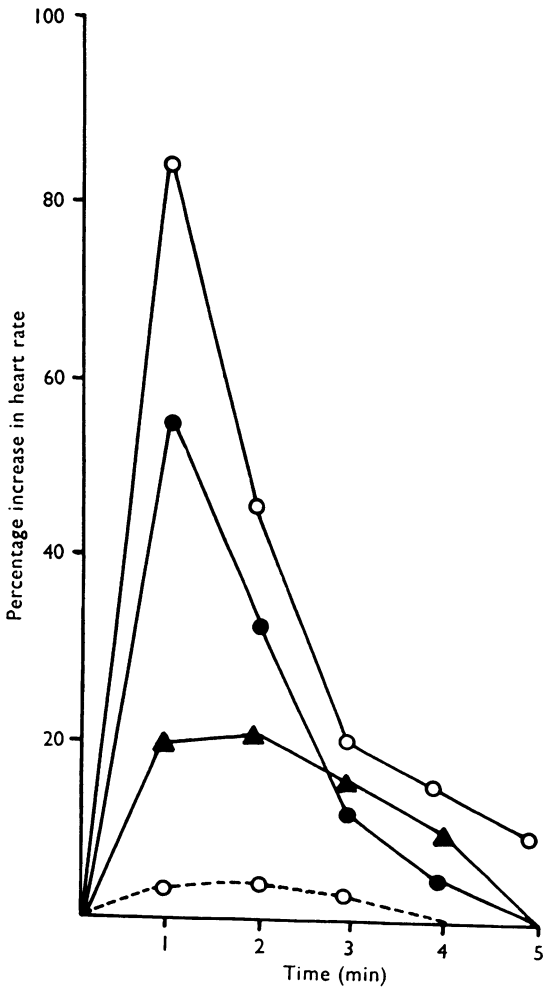

FIG. 4. Percentage increase in heart rate produced by intranodal injections in the dog of 5 μg of acetylcholine (●——●), 10 μg of cocaine (▲——▲), 5 μg of acetylcholine after 10 μg of cocaine (○——○), and 5 μg of acetylcholine after 100 μg of pronethalol (○- - -○), plotted against time after injection.

Blockade and potentiation of the S.A. node response to acetylcholine

A total of twelve experiments were performed in this series.

Dichloro-isopropylnoradrenaline (100 μg) or pronethalol (100 μg) injected intranodally blocked the positive chronotropic effects of intranodal injections of acetylcholine (Fig. 4), adrenaline or noradrenaline.

Cocaine hydrochloride (10 μg) given by the same route produced cardio-acceleration, and also potentiated the positive chronotropic response to 5 μg of acetylcholine (Fig. 4).

In reserpinized dogs, intranodal injections of 2, 5 or 10 μg of acetylcholine produced marked bradycardia, and in some instances a mild degree of heart block. Intranodal infusion of either 60 μg of adrenaline or 60 μg of noradrenaline in reserpinized animals restored the positive chronotropic response to acetylcholine (Fig. 5). The restoration of the response was of short duration, and subsequent injections of acetylcholine again produced bradycardia.

The intranodal injection of atropine, in 50 or 100 μg doses, blocked the negative as well as the positive chronotropic responses to acetylcholine.

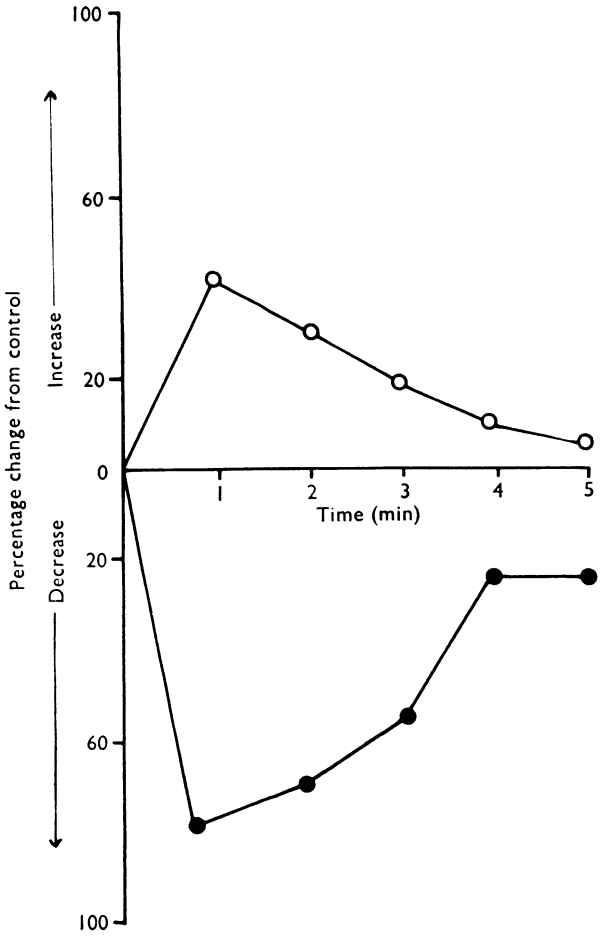

FIG. 5. Effects of the intranodal injection of 5 μg of acetylcholine in a dog previously injected with reserpine (●——●), and the effect of a similar injection after perfusion of the node with noradrenaline solution (○——○), plotted against time.

Discussion

The direct negative chronotropic and inotropic action of acetylcholine is well known, but it has been suggested that acetylcholine may also have a direct positive inotropic action on the heart (Buccino, Sonnenblick, Cooper & Braunwald, 1966).

It has also been reported that during ventricular arrest produced by acetylcholine there may be a feed-back stimulation, which results in atrial acceleration (Nadeau, Amir-Jahad & Roberge, 1967).

It has now been shown that acetylcholine, when applied to the S.A. node by close intra-arterial injection produces cardio-acceleration, similar to that produced by catecholamines. Further, the cardio-acceleration produced by acetylcholine was blocked by dichloro-isopropyl noradrenaline or pronethalol and potentiated by cocaine. In reserpinized animals, similar injections of acetylcholine resulted in bradycardia. When catecholamine stores were depleted in such animals by infusion of adrenaline, or noradrenaline, the excitatory effect of acetylcholine was temporarily restored.

The action of acetylcholine on the S.A. node appears to have two components. When injected directly into the nodal artery it may stimulate the parasympathetic system and also activate the sympathetic mechanism, but the latter action seems to predominate. When catecholamine stores are depleted the parasympathetic effect is unmasked.

It may be concluded that in the experimental conditions described acetylcholine activates adrenergic systems in the S.A. node, as has been proposed for other sites by Burn & Rand (1962).

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