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A hyoscine-resistant contraction of isolated chicken oesophagus in response to stimulation of parasympathetic nerves

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1. Contractions of the chicken isolated oesophagus or separated external muscle produced by stimulation of the vagus and descending oesophageal nerves were abolished by hyoscine (1–100 μg/ml.) if the duration of stimulation was less than 5 sec, but prolonged stimulation produced delayed contractions not antagonized by hyoscine.

2. The contractions to nerve stimulation were abolished by nerve section but not by bretylium, hexamethonium or tubocurarine.

3. In decerebrate chickens, intravenous hyoscine abolished the contractions of the oesophagus produced by nerve stimulation, but previous intravenous injection of hyoscine into chicks did not prevent subsequently isolated oesophageal preparations from contracting to nerve stimulation.

4. Prolonged nerve stimulation of an isolated oesophageal preparation did not produce a contraction from a piece of isolated guinea-pig ileum or post-crop chick oesophagus suspended in the same organ-bath.

5. It seems possible that small amounts of a slow contracting substance were released from the stimulated nerves together with acetylcholine.

In the anaesthetized fowl contractions of the oesophagus, crop, proventriculus and gizzard produced by stimulation of the vagus and descending oesophageal nerves are abolished by hyoscine or atropine (Hassan, 1967). The present paper describes experiments in which the isolated oesophagus responded to stimulation of these nerves with contractions resisting complete block by hyoscine. A preliminary report of the pharmacological characteristics of the hyoscine-resistant contraction of the chicken isolated oesophagus was communicated to the British and Scandinavian Pharmacological Societies Summer Meeting; it was concluded that hyoscine added to the organ-bath did not reach receptors responding to acetylcholine released from the nerves (Hassan, 1968). Further evidence suggests, however, that a slow contracting substance is released from the nerves together with acetylcholine, thus accounting for the resistance of the contraction to complete block by hyoscine.

Methods

Isolated preparations

Chicks aged 1–14 days (Brown Leghorn) were killed by an air embolus. An isolated preparation of pre-crop oesophagus with vagus and descending oesophageal nerves attached was made as described by Bartlet & Hassan (1968a). Sometimes a preparation of separated external muscle with nerve attached was made by everting a nerve-oesophagus preparation on a glass rod, peeling away the mucosa and reverting the muscularis externa. The preparations were suspended in Krebs solution and longitudinal contractions in response to nerve stimulation were recorded isotonically as previously described (Bartlet & Hassan, 1968a). Occasionally the post-crop oesophagus or a piece of guinea-pig ileum was suspended in the same organ-bath as the nerve-oesophagus. The vagus and descending oesophageal nerves were usually encircled by the same electrodes and stimulated together; and the term "nerve stimulation" in the text always refers to the synchronous stimulation of the two nerves. In some experiments the vagus or descending oesophageal nerve was freed from other tissues and stimulated separately; in referring to these experiments the terms "vagal stimulation" and "descending oesophageal nerve stimulation" were used.

Unless otherwise specified, the nerves were stimulated with a train of square wave pulses: width 10 msec, frequency 20 c/s and intensity 5 V, which usually produced maximal responses. Trains of stimuli were applied for periods varying from 3 to 90 sec in every 2 to 15 min.

Decerebrate preparations

Cockerels were lightly anaesthetized with halothane and the skull quickly trephined over the cerebrum which was then sucked out with a pump and replaced by cotton gauze to stop bleeding. After an interval for 60–90 min, oesophageal contractions were recorded by a balloon-tambour system (Hassan, 1967). The parameters of the stimuli applied to the vagus and descending oesophageal nerve were the same as for the *in vitro* preparations. A jugular vein was cannulated for the injection of drugs.

Drugs

The drugs used were: acetylcholine chloride, atropine sulphate, bretylium tosylate, hexamethonium bromide, 5-hydroxytryptamine creatinine sulphate, hyoscine hydrobromide, pentobarbitone sodium and tubocurarine chloride. Bretylium was kindly supplied by Mr. J. Roberts, Burroughs Wellcome & Co. Quantities of drugs in the text and figures refer to the salts.

Results

Isolated preparations

Nerve stimulation

Stimulation of the vagus and descending oesophageal nerves, separately or together, produced a contraction of the oesophagus within 1–2 sec of application of the stimulus. On prolonged stimulation, the preparation contracted for 10–20 sec

and then partially relaxed to about half of the initial response. Sometimes the response remained at this reduced level with increased rhythmicity superimposed (Fig. 1); in other preparations a second smaller contraction developed. After cessation of stimulation, relaxation was complete within 15 sec. The responses were reproducible for more than 2 hr when the nerve was stimulated for 90 sec at intervals of not less than 5 min.

Nerve transection and bretylium

To ascertain that direct stimulation of the oesophageal wall did not contribute to the response of the oesophagus to nerve stimulation, the nerves were cut near the oesophagus and the stimulation repeated. After cutting the nerves, the preparation no longer contracted to nerve stimulation whereas it still contracted to 5-hydroxytryptamine or to potassium chloride. Cocaine was previously shown to abolish the contraction of the oesophagus in response to prolonged supramaximal vagal stimulation (Bartlet & Hassan, 1968b), but in five of the present experiments bretylium (10 μg/ml.) did not affect it.

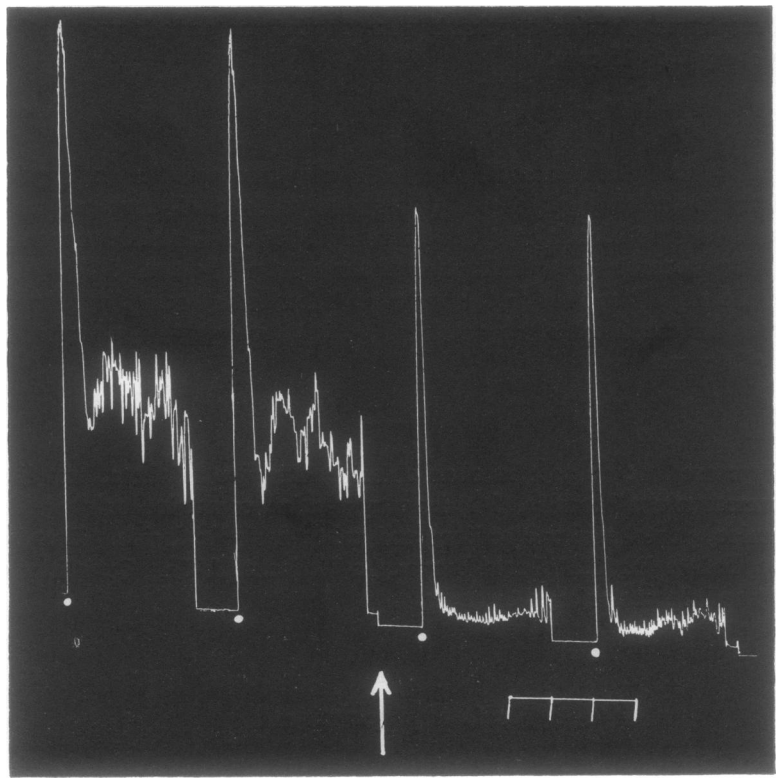

FIG. 1. Longitudinal contractions of chick isolated oesophagus. The dots mark nerve stimulation for 3 min in every 15 min. From the arrow to the end of the tracing hyoscine (100 μg/ml.) was present. Note hyoscine-resistant contraction to nerve stimulation and abolition of the second rise in tone by hyoscine. Time, 1 min.

Hyoscine and atropine

Exposure of the oesophagus to hyoscine or atropine (1 μg/ml. or more for 30 min) abolished the contraction to nerve stimulation when the period of stimulation was less than 5 sec but not when the nerves were stimulated for longer periods. Hyoscine was no more effective even when its concentration was raised to 100 μg/ml. The resistance of the contraction to hyoscine or atropine blockade was also observed in five experiments when the nerves were stimulated with 1 msec pulses at 10 c/s instead of with the usual stimulus parameters (see **Methods**). When the effect of hyoscine became steady the preparation contracted to nerve stimulation after a delay of 4–7 sec, the contraction rapidly reaching a peak (Fig. 2) which was usually lower than that of the contraction in the absence of hyoscine. On application of a prolonged train of stimuli, the hyoscine-resistant contraction was only maintained for about 10 sec, the preparation relaxing to the baseline without exhibiting a sustained high tone or second contraction as observed in the absence of hyoscine (Fig. 1). The response to vagal stimulation in the presence of hyoscine was not as steadily reproducible as that produced by stimulation of the descending oesophageal nerve or the two nerves together.

On three isolated oesophageal preparations made from 10 month old cockerels, hyoscine (100 μg/ml.) abolished only the contraction to short-duration nerve stimulation, a hyoscine-resistant contraction being produced on prolonged stimulation.

To ascertain whether hyoscine reached the receptors responding to nerve stimulation, a thinner preparation of separated external muscle with nerve attached was made. The contractions to nerve stimulation resisted complete block by hyoscine

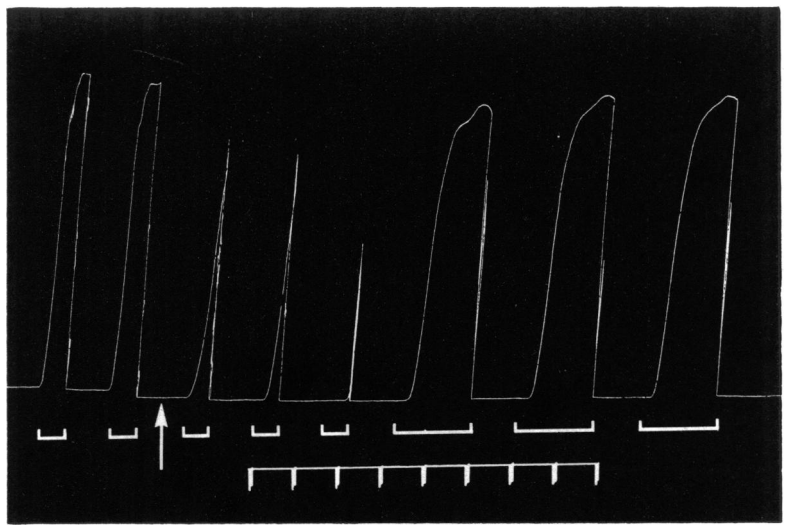

FIG. 2. Longitudinal contractions of chick isolated oesophagus produced by nerve stimulation for 3 sec (small brackets) or 9 sec (wider brackets) every 10 min. From the arrow to the end of the tracing hyoscine (10 μg/ml.) was present. Hyoscine abolished the contraction to 3 sec nerve stimulation but a hyoscine-resistant contraction was produced by the 9 sec stimulation. Time, 5 sec.

(100 μg/ml.) (four experiments). Furthermore, when hyoscine (100 μg/g) was injected intravenously into six chicks 5 to 10 min before making isolated nerve-oesophagus preparations; the preparations contracted to nerve stimulation but acetylcholine (10 μg/ml.) did not produce contractions until 1 hr or more had elapsed. Three of these chicks had been anaesthetized with halothane and a fourth was killed with halothane before making the nerve-oesophagus preparations.

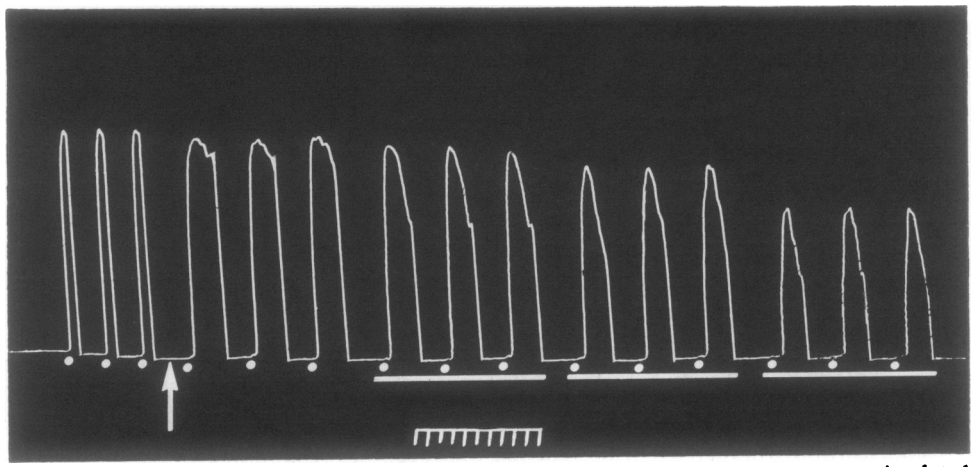

FIG. 3. Longitudinal contractions of chick isolated oesophagus. The nerves were stimulated at 10 min intervals, the dots marking 5 sec stimulation before the arrow and 15 sec after it. From the arrow to the end of the tracing hyoscine (100 μg/ml.) was present. The white bars mark the presence of hexamethonium, 1, 10 and 100 μg/ml., respectively. Hexamethonium did not abolish the hyoscine-resistant contraction of the oesophagus to nerve stimulation. Time, 5 sec.

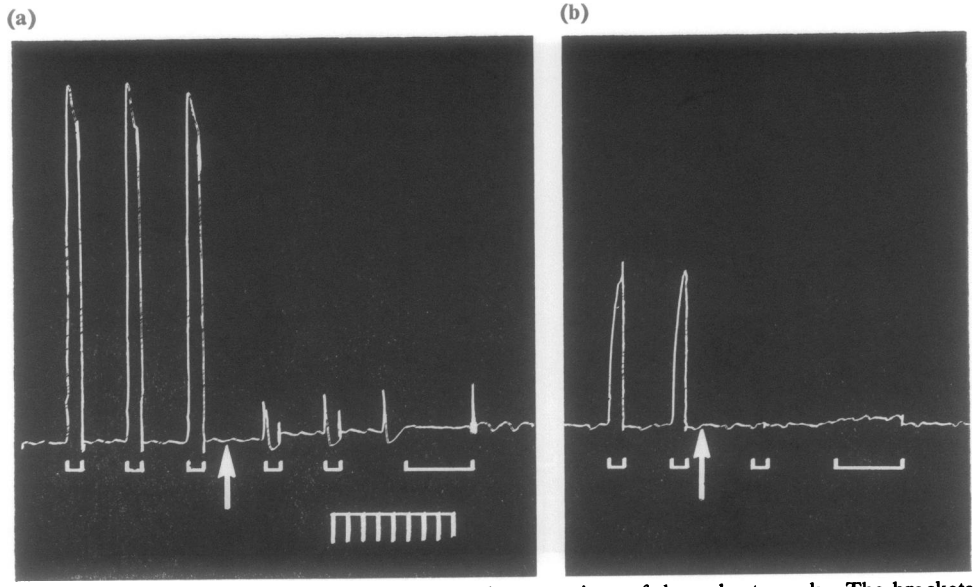

FIG. 4. Balloon-tambour record of oesophageal contractions of decerebrate cock. The brackets mark stimulation of the descending oesophageal nerve at 5 min intervals. At the arrows hyoscine (100 μg/kg) was injected intravenously; 2 hr elapsed between (a) and (b). Hyoscine abolished the response of the oesophagus to stimulation of the descending oesophageal nerve. Time, 5 sec.

In five experiments, isolated oesophageal preparations were made to contract to nerve stimulation in the presence of hyoscine (100 μg/ml.), the stimulus being applied continuously for 30 min. In three of these experiments an isolated guinea-pig ileum, and in two of them a post-crop chick oesophagus suspended in the same organ-bath as the nerve-oesophagus preparation, exhibited no response during this prolonged period of nerve stimulation.

Hexamethonium and tubocurarine

Hexamethonium (5 μg/ml.) did not appreciably antagonize the contractions to nerve stimulation, but in six experiments hexamethonium, in a concentration of 50 μg/ml., reduced the height of the contractions by a mean of $8{\cdot}5 \pm 3{\cdot}0\%$ ($P<0{\cdot}05$). This mean includes the results of two experiments in which hexamethonium was without effect.

In the presence of hyoscine, hexamethonium (1 or 10 μg/ml.) did not produce a significant inhibition of the contraction of the oesophagus to nerve stimulation, but in a concentration of 100 μg/ml. hexamethonium inhibited the response by $59 \pm 14\%$ (four experiments, $P<0{\cdot}05$) (Fig. 3).

Tubocurarine (5 or 50 μg/ml.) added to the organ-bath for 30 min did not antagonize the response of the oesophagus to nerve stimulation (two experiments in each instance), but in the presence of hyoscine, tubocurarine (50 μg/ml.) reduced the height of the hyoscine-resistant contraction by $21 \pm 8\%$ (six experiments, $P<0{\cdot}05$).

In vivo preparations

In decerebrate chickens, contractions of the oesophagus to vagal and descending oesophageal nerve stimulation were abolished by intravenous injection of hyoscine (100 μg/kg) (four experiments) even when the duration of stimulation was as long as 30 or 45 sec (Fig. 4). In one of these experiments the administration of pentobarbitone (30 mg/kg) did not affect the oesophageal contraction to stimulation of the descending oesophageal nerve which was subsequently abolished by hyoscine (100 μg/kg).

Discussion

The contractions of the isolated oesophagus to nerve stimulation in the presence of hyoscine or the ganglion-blocking drugs could not have been due to passage of current through the plain muscle of the jugular vein which was encircled by the stimulating electrodes because a hyoscine-resistant contraction was observed on stimulation of either nerve dissected free from contiguous tissue. Nor could the effect have been due to passage of current through the bathing solution, for no response was obtained after cutting the nerves or after cocaine (Bartlet & Hassan, 1968b). The response did not seem to be due to stimulation of adrenergic fibres for it was not affected by bretylium.

Contractions of the oesophagus to nerve stimulation were abolished by intravenous hyoscine in decerebrate chickens which had been allowed at least 1 hr after brief anaesthesia with halothane for the anaesthetic to be removed. Thus it seems improbable that the general anaesthetic, sodium pentobarbitone, masked a hyoscine-resistant response to nerve stimulation in the previous *in vivo* experiments of Hassan (1967).

Hyoscine-resistant contractions were obtained from isolated oesophageal preparations made from the adult fowl, showing that a difference in age of the birds could not account for the difference in effectiveness of hyoscine in the *in vitro* and *in vivo* experiments.

It is improbable that the slow hyoscine-resistant contraction of the oseophagus to nerve stimulation was produced by direct electrical coupling, since ephaptic transmission is very rapid (Martin & Pilar, 1963). A more plausible possibility is that hyoscine did not reach all the receptors in the isolated chicken oesophagus; it has been shown that some drugs only combine with receptors in the muscularis mucosae of isolated guinea-pig oesophagus after separation of the external muscle from the mucosa (Bartlet, 1968a, b). However, hyoscine abolished the contractions of the chick oesophagus produced by physostigmine, which apparently stimulated neural structures, since its action was blocked by cocaine (Bartlet & Hassan, 1968b). Thus, in these experiments, hyoscine seemed effective in antagonizing acetylcholine released from neural structures. However, in the present experiments, hyoscine did not abolish the contractions to nerve stimulation of a thin separated external muscle preparation made from the chick oesophagus. Furthermore, intravenous injection of large doses of hyoscine into chicks before isolation of the nerve-oesophagus preparation was similarly ineffective in abolishing the contraction to nerve stimulation although the action of acetylcholine was blocked. Thus it is hard to reconcile these results with the assumption that hyoscine failed to reach all the receptors responding to acetylcholine.

It seemed possible that the hyoscine-resistant contraction was due to the release of a slow contracting substance from the nerves together with acetylcholine. A similar view has recently been put forward by Ambache & Freeman (1969) from their experiments with the separated longitudinal muscle of guinea-pig ileum stimulated transmurally. It might be that *in vivo* a slow contracting substance could be removed from the vicinity of the nerve terminals by the circulation before it contracted the oesophagus. *In vitro*, a slow contracting substance released from nerves would only be removed by diffusion and/or metabolism, and if these effects occurred slowly, the substance might accumulate in sufficient amount to produce a contraction. However, pieces of guinea-pig ileum or chick oesophagus did not contract when suspended in the same organ-bath as the nerve stimulated chick oesophagus, so that no slow-contracting substance could be detected in this way.

The inability of hexamethonium and tubocurarine to abolish the contractions of the oesophagus produced by nerve stimulation *in vitro* was also observed *in vivo* (Hassan, 1967). Since ganglia can be demonstrated histologically in the wall of the chick oesophagus (Bartlet & Hassan, 1968b), the failure of these ganglion-blocking drugs to block the effects of nerve stimulation is not attributable to absence of ganglia in the preparations. It is possible that the stimulated nerve fibres do not relay with post-synaptic fibres at ganglionic level in the chick oesophagus; the vagal fibres innervating the external muscle of guinea-pig oesophagus do not synapse with the intramural ganglia of Auerbach's plexus (Bartlet, 1968c). Alternatively transmission at the synapses may be effected by a substance not having a nicotinic action, or, hexamethonium and tubocurarine may not reach ganglionic receptors in the preparations.

According to Bowman & Everett (1964), atropine (0·02 μg/ml. and above)

abolished the contractions of the chick isolated oesophagus to parasympathetic stimulation. In their experiments the nerves were stimulated with rectangular pulses of 0·5 or 1 msec duration, whereas in the present experiments 10 msec pulses were usually used. However this difference in pulse width does not account for the difference in block by atropine, for, in some of the present experiments in which the pulse width was reduced to 1 msec duration, an atropine-resistant contraction was still obtained. In the experiments of Bowman & Everett (1964) hexamethonium (2–4 μg/ml.) and other drugs which block ganglia produced a 50–80% depression of the height of contraction of the chick isolated oesophagus produced by vagal stimulation. Moreover, Everett (1966) assumed that the addition of hexamethonium (4 μg/ml.) to the Krebs solution bathing the oesophagus restricted the effective transmural stimulation to the postganglionic fibres. This is at variance with the present results where hexamethonium in a concentration of less than 50 μg/ml. failed to produce a significant antagonism of the response to vagal stimulation. No plausible explanation can be suggested for these differences in the actions of hexamethonium and atropine as observed by Bowman & Everett (1964) and in the present experiments.

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