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A NOTE ON THE ISOLATED NERVE STERNO-TRACHEALIS PREPARATION OF THE CHICKEN

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A new avian neuromuscular preparation of the sterno-trachealis muscle of the domestic chicken is described. The pharmacology of common autonomic and neuromuscular blocking drugs is given. The preparation is useful for mode of action experiments with depolarizing drugs. A short description of the anatomy is also given.

During the course of a study of the pharmacology of the trachea in various animals, an avian trachea, that of the chicken, was studied by the classical method of tracheal chains (Castillo & De Beer, 1947) without success, no responses being obtained to spasmogens or spasmolytics of any kind. As would be expected from the complete cartilaginous rings in avians, a circular smooth muscle component is absent. On recording the changes in volume of the trachea during transmural stimulation by a capillary manometer method (Foster, 1960), no change of volume could be detected while the trachea was held at constant length. However, it was found that a well-defined longitudinal musculature was present which appears to be of typically avian skeletal muscle kind, possessing both a "slow" and a "fast" system. Further study has revealed that the chicken trachea possesses a specialized musculature with an obvious innervation from which useful neuromuscular preparations may be easily made. These may have value both as demonstrations of avian muscle and, because of their often high sensitivity to depolarizing neuromuscular blocking agents, in the assay of these substances.

The object of this note is to point out the pertinent features of general anatomy, myology and innervation of the trachea and to outline something of its general pharmacology and its possible use in the study of depolarizing neuromuscular blocking agents.

Anatomy. The anatomy of the chicken trachea is represented diagrammatically in Fig. 1. The cartilaginous rings are complete as may be shown histologically and by maceration, and are connected by elastic tissue. The rings are so shaped in section that they may slide past one another, allowing remarkable powers of shortening. Situated on the outside of the rings down each side of the trachea are two muscle bands, each some 2.5 to 3.5 mm in width and some 0.1 to 0.3 mm in thickness. On the surface of the muscle lies a prominent blood vessel and a nerve: both give off branches to the muscle and trachea proper. The muscles are

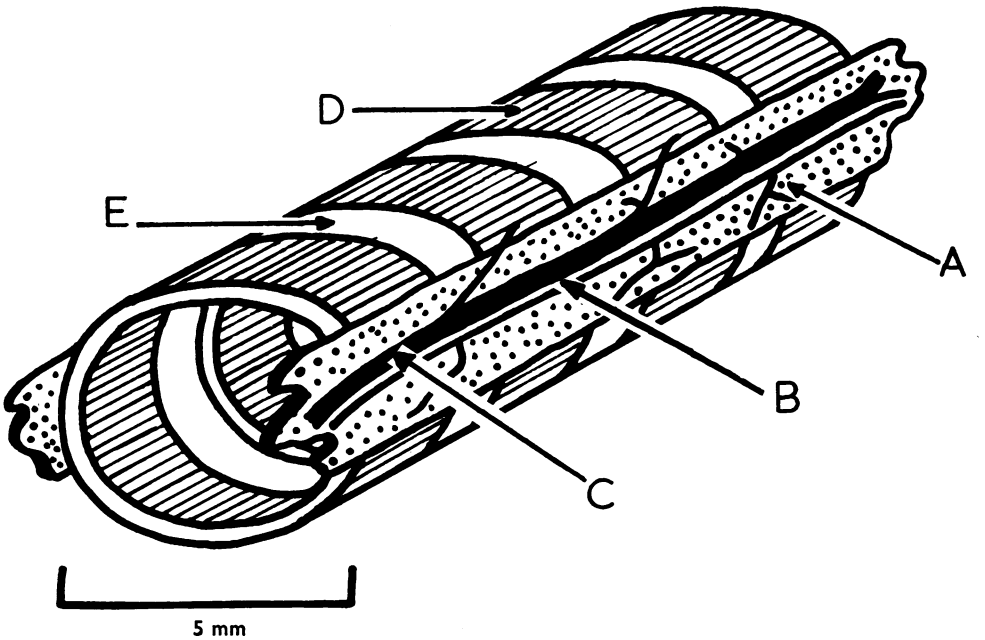

Fig. 1. Chicken trachea. Diagram to show general anatomy, myology and innervation. A, Lateral muscle strip. B, Principal nerve of trachea. C, Principal blood supply of trachea. D, Complete cartilaginous ring. E, Elastic connective tissue.

continuous cranially with a pair of thin muscles of the laryngeal region. Some 20 mm above the tracheal bifurcation, caudally, the muscle leaves the trachea and passes outwards to be inserted into the sternum, there being no apparent extension to the region of the tracheal bifurcation. The muscle appears to receive its nerve supply mainly from elements of the vago-sympathetic trunk and from the first cervical nerve. It is derived developmentally from the internal fasciculus of the rectus cervicus, a division of the hypobranchial muscles, so that its motor fibres are probably derived from the hypoglossal nerve and the first and second cervical nerves (Edgeworth, 1935). Also a variable number of nerves reach the trachea from the oesophagus. The nerve lying on the muscle is probably both autonomic and somatic motor in function.

No clear description or name for this chicken muscle has been traced in the standard texts ; from its anatomy and by analogy with related muscles described in other birds by Edgeworth (1935), I have provisionally called it the sterno-trachealis muscle.

Histological examination shows the muscle to be typically striated muscle. The rest of the trachea seems to be devoid of striated or smooth musculature, an observation which is borne out by the experimental evidence.

METHODS

Chickens, cocks or hens of 7 to 12 weeks have been used. Tracheas were taken from animals under pentobarbitone sodium anaesthesia or from animals killed by stunning or

decapitation. No differences have been noted in preparations taken from animals treated in any of these ways.

The whole length of the trachea is removed and placed in cold oxygenated Krebs solution and the lower 20 mm discarded. The trachea is carefully cleared of adhering connective tissue, so exposing the delicate muscle and its nerve supply. The trachea is now gently stretched and a length of some 4 cm removed and cut along its length between the muscle bands, so making two preparations. The "half trachea" is then gently pinned out, muscle uppermost, and some 8 to 10 mm of nerve dissected free. The nerve muscle preparation may be arranged for nerve and muscle stimulation in the usual way, the preparation being secured by threads passed through the cartilaginous part of the trachea, so leaving the muscle undamaged. The preparation is then placed in Krebs solution at 38° to 40° C bubbled with 95% O_2 and 5% CO_2. Recording may be semi-isometric or isotonic. To demonstrate the "slow" contracture response to best advantage a light balsa frontal writing lever is preferred, the load being 0.8 to 1.6 g and magnification some 10 to 15 times.

When studying contracture responses a contact time of 90 to 120 sec and a cycle of 5 to 8 min may be used.

All drug concentrations are given as final bath concentration in g/ml. of base. Typical electrical stimulation parameters used were: supramaximal nerve stimulation at 20 to 40 V, duration 30 to 100 μsec; direct muscle stimulation 40 to 60 V, duration 1 msec.

RESULTS

Nerve stimulation. Stimulation at rates of from 5/min to 180/min gave well-maintained twitches. At rates of 180 to 1,500/min tetanus developed. A rate of stimulation of 5 to 15/min was most commonly used. Viability at these rates is satisfactory; an average preparation gives good responses for some 5 to 8 hr.

Drug responses. The response to nerve stimulation is abolished by 2.5×10^{-6} D-tubocurarine or gallamine, the direct muscle response being unaffected. The actions of D-tubocurarine and gallamine are readily reversed by washing and both are antagonized by 5×10^{-7} eserine or neostigmine. Atropine and hexamethonium at 5×10^{-5} have no effect on the twitch response.

Acetylcholine. The response is variable both in relation to threshold dose, time of onset and rate of contracture. The threshold may be as low as 2×10^{-7}, but is often far higher. The sensitivity to acetylcholine may be increased 8 to 16 times by 5×10^{-7} eserine or neostigmine.

Decamethonium, suxamethonium, nicotine, carbachol and tetramethylammonium. All these drugs gave contracture responses and a dose-dependent degree of neuromuscular block.

The sensitivity to decamethonium and suxamethonium is high; as little as 2.5×10^{-8} decamethonium and 1×10^{-7} suxamethonium may be detected with a sensitive preparation.

Good responses to carbachol, nicotine and tetramethylammonium can also be obtained.

Table 1 gives the mean values for drug concentrations giving a 50% maximal contracture. All the drugs shown give the same maximum contracture except acetylcholine and methacholine. The responses to acetylcholine are variable and often require high concentrations. Thus, of ten preparations, one was obtained in

TABLE 1

MEAN DRUG CONCENTRATION ± STANDARD ERROR (G/ML.) GIVING A RESPONSE 50% OF MAXIMUM. THE FIGURES IN PARENTHESIS ARE THE NUMBER OF FOWLS USED

Decamethonium	$0{\cdot}204 \pm 0{\cdot}072 \times 10^{-6}$ (10)
Suxamethonium	$0{\cdot}653 \pm 0{\cdot}285 \times 10^{-6}$ (10)
Nicotine	$1{\cdot}59 \pm 0{\cdot}298 \times 10^{-6}$ (10)
Carbachol	$2{\cdot}89 \pm 0{\cdot}308 \times 10^{-6}$ (10)
Tetramethylammonium	$8{\cdot}80 \pm 0{\cdot}440 \times 10^{-6}$ (10)
Acetylcholine	$19{\cdot}76 \pm 2{\cdot}03 \times 10^{-6}$ (9)
Methacholine	$143{\cdot}0 \pm 16{\cdot}35 \times 10^{-6}$ (6)

which the maximal contracture was less than the 50% responses to the other drugs. Even less sensitivity was seen with methacholine, for in only six of the ten preparations were the maximal contractures greater than those seen with the other drugs, and then only with high concentrations.

The sensitivity of a good preparation to decamethonium and suxamethonium is similar to that of the chick biventer cervicus (Ginsborg & Warriner, 1960) and the chick semispinalis muscle (Child & Zaimis, 1960). Also it has been found that the preparation has high resistance to desensitization by repeated doses of decamethonium or suxamethonium and is probably better in this respect than the two preparations mentioned above. Fig. 2 shows responses to increasing doses of decamethonium and suxamethonium and equipotent doses of suxamethonium and decamethonium repeated over a period of 4 hr, there being no apparent desensitization. This property may make the sterno-trachealis useful in the assay of these drugs.

Neostigmine and eserine (5×10^{-7}) give an increase in the response to indirect stimulation and an increased sensitivity to acetylcholine. Higher doses sometimes

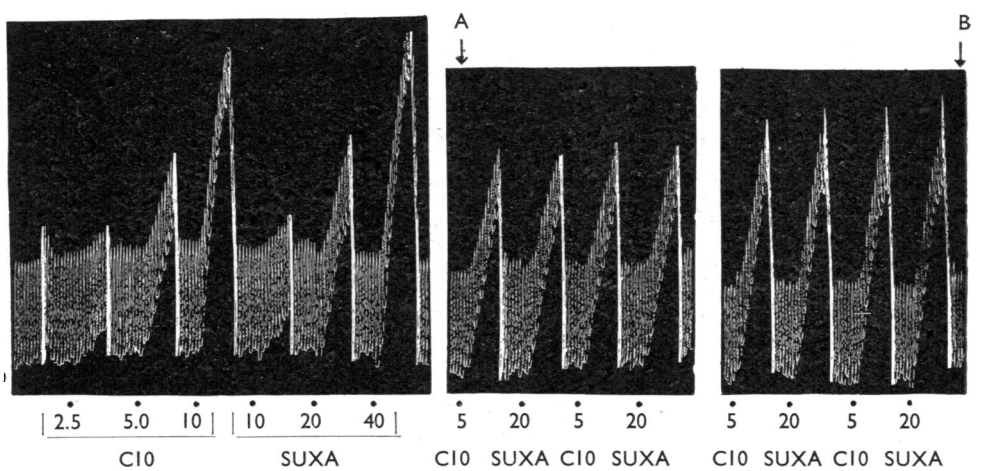

Fig. 2. Tracing showing responses to decamethonium (C10) and suxamethonium (SUXA) and reproducibility of response. Between A and B alternate doses of C10 and SUXA were administered over a period of 4 hr. Drug contact time 100 sec, 6 min cycle. Drug concentrations as $g \times 10^{-8}$/ml. of bath fluid. Nerve stimulation : 5/min.

give slow contractures. *Histamine* (5×10^{-5}), *5-hydroxytryptamine* (5×10^{-5}) and *vasopressin* (0.4 u./ml.) have no effect on the twitch or contracture responses; *adrenaline* (5×10^{-6}) gives a variable increase in the response to indirect stimulation.

If the sterno-trachealis is carefully dissected from the trachea the remaining structure is inert to all the drugs and procedures described above: this confirms the histological evidence that the sterno-trachealis is the principal muscle of the trachea.

DISCUSSION

The results indicate that the response to drugs of the sterno-trachealis muscle of the fowl resembles that of skeletal muscle: it is very sensitive to the action of neuromuscular blocking agents and is insensitive to such typical smooth muscle stimulants as histamine, 5-hydroxytryptamine and vasopressin.

The sterno-trachealis muscle may have advantages over previously described avian muscle preparations. Firstly, as many as 4 to 6 preparations may be obtained from one animal. Secondly, the preparation is simple to mount and viability is high. The high sensitivity which is often obtained to decamethonium and suxamethonium may be useful in assays. Many authors have noted the presence of a muscle running from the syrinx or from the sternum up the trachea and a function as an accessory muscle of vocalization has been suggested. In the chicken the muscle does not seem to be connected to the syrinx.

The muscle is said to be represented in some species by a fibrous band and in others to be hypertrophied, as in vocal birds like the parrot. The muscle may serve to alter the resonance qualities of the trachea or alternatively to adjust the trachea to changes in length of the neck.

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