Short communications

A technique for the study of muscle relaxants by stimulating the spinal motor nerve outflow in the pithed rat

A. S. CLANACHAN AND T. C. MUIR

Department of Pharmacology, The University, Glasgow, G12 8QQ

The motor nerve outflow in the pithed rat was stimulated from the spinal column and contractions of individual skeletal muscles recorded. The preparation is anaesthetic-free and well suited to a study of muscle relaxants.

Studies of muscle relaxants both *in vitro* and in whole animals, have been largely made by stimulating, electrically, an isolated exposed motor nerve and recording the response of the skeletal muscle innervated. In such whole animal preparations, the enforced use of anaesthetics may influence the responses to muscle relaxants (Foldes, 1959) while the operative interference in isolating, exposing and stimulating the motor nerves could affect the response of the skeletal muscle either directly or indirectly via the blood supply.

In the present technique, a gaseous anaesthetic is required only for a brief (4–5 min) period to permit pithing while the isolation and exposure of nerves are obviated. The pithed rat preparation (Gillespie, MacLaren & Pollock, 1970) has been used to permit localized stimulation of the spinal nerve roots at their source in the cord. Reproducible contractions of individual skeletal muscles can be obtained for long (5–6 h) periods and the preparation is convenient for the study of muscle relaxants.

Methods:—Rats (200–300 g) anaesthetized with a mixture of halothane (3·5%) in nitrous oxide and oxygen (3:1 v/v) were pithed, blood pressure recorded from one common carotid artery and one external jugular vein cannulated. The pithing electrode assembly could be moved to any desired level within the spinal canal while the indifferent electrode, a length (3 cm) of silver wire, was inserted subcutaneously in the lumbar region parallel to the spine. The spinal nerve roots were stimulated using single, supramaximal (1 ms) pulses usually at 1 Hz (Gillespie, *et al.*, 1970). Temperature was monitored rectally and maintained at 37° C by a tungsten lamp. The tendons of the anterior tibialis, soleus and gastrocnemius muscles were isolated separately. Tension recordings were made by connecting the appropriate isolated tendon to an isometric strain gauge. The limb from which recordings were made was immobilized by a clamped stainless steel drill passed through the femur close to the knee. The leg below the knee was held horizontally by a clamp around the foot. An initial (10 g) tension was applied to the muscle being studied. Movement of the muscles of the contralateral limb and the trunk, also stimulated by the spinal electrode, was minimized by pinning the skin to the dissecting board.

With the exception of halothane (I.C.I.), drugs were dissolved in saline (0·9% NaCl w/v) and doses refer to the salts; decamethonium chloride (Allen & Hanbury), gallamine triethiodide (May & Baker), neostigmine bromide (Roche), pancuronium bromide (kindly donated by the manufacturers, Organon), suxamethonium chloride (Allen & Hanbury), (+)–tubocurarine chloride (Burroughs Wellcome).

Results.—To obtain discrete stimulation of the skeletal muscles of the lower limb the optimum position for the electrode, confirmed by X-ray photography, was L5-6 (cf. Greene, 1935). The smallest length of electrode which could stimulate outflows from both dermatomes was 1·5 cm. Reproducible twitches (e.g., 120–150 g tension for gastrocnemius) could be obtained to single pulses at 0·1 Hz but rapidly waned at 1 Hz or above. Twitch height recovered when stimulation at 0·1 Hz was resumed.

The principal characteristics of both non-depolarizing and depolarizing muscle relaxants were easily demonstrated (Fig. 1). Inhibition of twitch height produced by tubocurarine (0·04 mg/kg) increased with repeated administration but became reproducible after 3–4 doses; it was rapid in onset (maximum block obtained in 2–3 min) and virtual recovery from approximately 85% block took 15 minutes. Tubocurarine block was additive

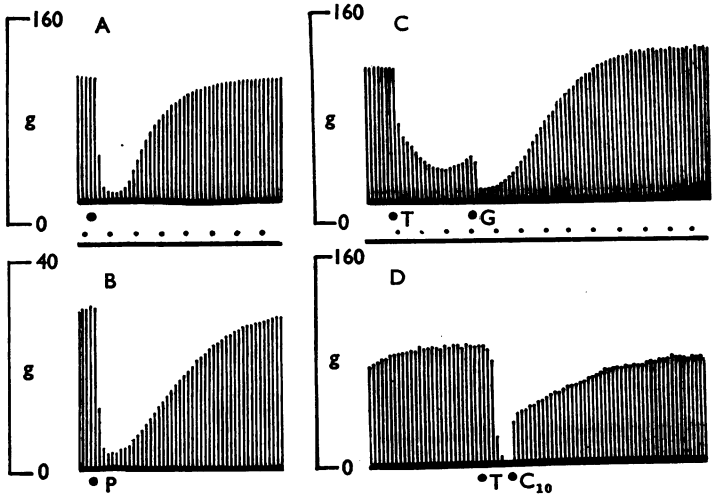

FIG. 1. The effect of muscle relaxants on the response of the skeletal muscles to supramaximal stimulation (0·1 Hz) of the spinal motor outflow in the pithed rat. In A and B in a rat of 200 g, the sensitivity of the simultaneously recorded contractions of the tibialis (A) and soleus (B) muscles to pancuronium (P, 0·08 mg/kg) is approximately similar. At C and D respectively in another rat (200 g), tubocurarine (T, 0·04 mg/kg) block was enhanced by gallamine (G, 0·04 mg/kg) and antagonized by decamethonium (C_{10}, 0·08 mg/kg). The ordinate scale refers to the tension (g) developed; the horizontal time marker between the traces occurs at 1 min intervals.

with that of other non-depolarizers (gallamine 0·04 mg/kg, Fig. 1C), reversed by anticholinesterases and antagonized by depolarizing relaxants (Fig. 1D).

Decamethonium in sub-blocking doses (0·80 mg/kg) enhanced twitch height, and in larger doses (1·2 mg/kg) like suxamethonium (0·2 mg/kg) produced dual block (Zaimis, 1953). The initial depolarization, as shown by an enhancement of twitch height, changed to a block characteristic of that of non-depolarizing relaxants which was reversed by neostigmine. In consequence of dual block, the action of decamethonium was not reversed by non-depolarizing drugs. The effects of both depolarizing and non-depolarizing drugs were compared on the responses of the anterior tibialis (fast muscle fibres) and soleus muscles (slow muscle fibres), see Fig. 1A and 1B. The sensitivity of each muscle to either class of relaxant was approximately similar. In other mammalian species, e.g., cat, the anterior tibialis shows a greater sensitivity to depolarizing compared with non-depolarizing drugs while the sensitivity of the soleus muscle to both types of relaxants is comparable.

While a gaseous anaesthetic was employed for a brief (3 min) period to permit pithing, the effects disappear and transmission both at the ganglion and neuroeffector junctions remains uninhibited (Gillespie & Muir, 1967, Gillespie *et al.*, 1970). The present preparation is therefore anaesthetic-free and suited to a study of the interaction between anaesthetics and muscle relaxants. Halothane (1%) administered via the input of the respiratory pump, lowered blood pressure and increased twitch tension of the gastrocnemius muscle. Halothane is known to increase twitch tension in humans (Baraka, 1968). Both tubocurarine and suxamethonium block were enhanced and prolonged by halothane.

Discussion.—In this preparation no operative interference is required to expose the peripheral nerves in order to stimulate them. The functional integrity of the nerves remains intact. This allows more reproducible conditions of stimulation because the nerve trunk is not exposed to drying or other damage. Since the electrode is firmly fixed in the spinal vertebrae, uncontrolled movement between the nervous outflow and the electrode, which might otherwise alter the threshold or the

number of nerve fibres being stimulated, is eliminated. The response of the skeletal muscles could be affected conceivably by changes in blood flow accompanying excitation of the sympathetic vasoconstrictor fibres in the hind limb. However, stimulation at L5–6 in the rat causes little change in either blood pressure or heart rate (Gillespie *et al.*, 1970). Although the parasympathetic is optimally stimulated at L5–6, this is unlikely to affect the response significantly and occurs only at frequencies greater than 1 Hz. In the present preparation, stimulation at frequencies above 1 Hz caused twitch height to wane rapidly making difficult a study of presynaptically active drugs (e.g., the hemicholiniums) whose mode of action is best characterized with high frequencies of stimulation (Schueler, 1960).

Although the rat is relatively insensitive to depolarizing drugs and exhibits dual block, the preparation can be used to distinguish between depolarizing and non-depolarizing muscle relaxants and appears suitable in screening procedures and for teaching and research purposes.

REFERENCES

BARAKA, A. (1968). Effect of halothane on d-tubocurarine and suxamethonium block in man. *Br. J. Anaesth.*, **40**, 602–606.

FOLDES, F. F. (1959). Factors which alter the effects of muscle relaxants. *Anesthesiology*, **20**, 464–502.

GILLESPIE, J. S. & MUIR, T. C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to the blood vessels in the pithed rat. *Br. J. Pharmac. Chemother.*, **30**, 78–87.

GILLESPIE, J. S., MACLAREN, A. & POLLOCK, D. (1970). A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. *Br. J. Pharmac.*, **40**, 257–267.

GREENE, E. C. (1935). *The Anatomy of the Rat.* New York: Hafner Publishing Co.

SCHUELER, F. W. (1960). Mechanisms of action of hemicholiniums. *Int. Rev. Neurobiol.*, **2**, 77–97.

ZAIMIS, E. J. (1953). Motor end-plate differences as a determining factor in the mode of action of blocking substances. *J. Physiol., Lond.*, **122**, 238–251.

(*Received July* 24, 1972)