SOME PHARMACOLOGICAL PROPERTIES OF MUREXINE (UROCANOYLCHOLINE)

BY

M. J. KEYL* AND V. P. WHITTAKER†

From the Department of Physiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio, U.S.A.

Murexine (urocanoylcholine, [2-β-imidazol-4(5)-ylacryloyloxyethyl] trimethylammonium bromide) has been shown to possess ganglion stimulating and neuromuscular blocking actions in cat, dog and rat. The blockade has been shown to be of the depolarizing type both on the basis of the resemblance of the pharmacological properties of the substance to that of known depolarizing blocking agents, and also directly by recording the depolarization produced in the endplate region of the gracilis muscle of the rat.

The recent appearance of several reports on the pharmacology of murexine (urocanoylcholine, [2-β-imidazol-4(5)-ylacryloyloxyethyl] trimethylammonium bromide) (Erspamer and Glässer, 1957; Grelis and Tabachnick, 1957; Quilliam, 1957) has prompted us to give a fuller account of some experiments carried out with this compound in 1955 which have so far been reported only in abstract form (Keyl and Whittaker, 1955).

The compound is of interest in being a naturally occurring choline ester. It was first identified by Erspamer and Benati (1953) in three Mediterranean species of whelks of the family Muricidae and later in three other North Atlantic Muricidae (Whittaker and Michaelson, 1954; Whittaker, 1955; Keyl, Michaelson, and Whittaker, 1957).

Our experiments confirm that murexine has a ganglion stimulating and neuromuscular blocking action. We have also demonstrated by means of the scanning electrode technique of Burns and Paton (1951) that, in the rat, the neuromuscular blockade is of the depolarizing type.

METHODS

Hydrolysis by Cholinesterases.—This was measured at 37° and *p*H 7.4 in bicarbonate buffer (0.023 M-NaHCO₃ in equilibrium with 95% N₂ and 5% CO₂ in the gas phase) using the Warburg manometric technique as modified by Ammon (1933). The following esterase preparations were used: purified bovine red cell cholinesterase (Winthrop Stearns); rat plasma; dog plasma; human plasma; Harvard

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*Present address: Department of Physiology, Medical School, University of Oklahoma, Oklahoma City, Okla., U.S.A.

†Present address: A.R.C. Institute of Animal Physiology, Babraham, Cambridge.

human plasma fraction IV-6-3 (kindly supplied by Dr. D. M. Surgenor and stabilized by addition of 1% gum acacia to the medium). The rate of hydrolysis of murexine is expressed as μl. CO_2 evolved from the buffer/mg. dry wt. of enzyme preparation/hr. (Q_{UrCh}), or as % of the corresponding rate with acetylcholine (Q_{ACh}).

Animal Experiments.—The effect of intravenous administration of murexine bromide on blood pressure, respiration and neuromuscular transmission was determined on 17 dogs and 6 cats. The cats were anaesthetized with 0.6 ml./kg. Dial with urethane solution (Ciba) and the dogs with 0.7 ml./kg. Dial with urethane solution or 30 mg./kg. pentobarbitone sodium. Respiration was recorded on a smoked drum by means of a tambour; blood pressure was similarly recorded by means of a mercury manometer connected to a cannula in the femoral artery. Heparin was used as anticoagulant. The effect of the drug on neuromuscular transmission was determined by recording isotonic contractions of the gastrocnemius muscles in response to supramaximal electrical stimulation once every 2 sec. through the sciatic nerve. Rectangular wave stimuli of 0.1 m.sec. duration and 10 to 20 V. strength were delivered by a Grass model 3C stimulator through shielded platinum electrodes 4 mm. apart placed on the nerve distally to a crushed region.

Scanning Electrode Technique.—Muscle action potentials and surface depolarization in the endplate region of the gracilis muscle of the rat were measured by means of the scanning electrode technique of Burns and Paton (1951). Potentials were led through cathode followers into a D.C. amplifier and recorded photographically after display on a double beam cathode ray tube. To facilitate the interpretation of the record, the beam recording action potentials was made to move in an axis at right-angles to that recording the endplate potentials.

RESULTS

Hydrolysis of Murexine by Cholinesterases

The rate of hydrolysis of murexine by representative mammalian cholinesterases is shown in Table I. It will be noted that the ester is split at a negligible rate by the acetocholinesterase of bovine red cells and by rat and dog plasma. It is split at approximately 7% of the acetylcholine

TABLE I

HYDROLYSIS OF MUREXINE BY CHOLINESTERASES

* Substrate concentration: red-cell preparation, 10 mM; others, 30 mM. † Substrate concentration: 10 mM. ‡ Calculated on assumption that the lyophilized preparation contains 3% enzyme preparation in NaCl-gelatine-phosphate stabilizer, as stated in Winthrop Stearns' specification.

Cholinesterase Preparation	Q_{ACh}*	Q_{UrCh}†	$\dfrac{Q_{UrCh}}{Q_{ACh}} \times 100$	% Inhibition by 10 μM. Eserine
Bovine red-cell (purified)	7.1×10^4‡	0	0	—
Rat plasma (unpurified)	6.4	0	0	—
Dog plasma (unpurified)	38	0	0	—
Human plasma (unpurified)	37	2.6	6.8	100
Human plasma fraction (IV-6-3)	6.2×10^4	4×10^3	7.0	—

rate by human plasma and by highly purified human plasma cholinesterase. The identity of the relative rates of hydrolysis of murexine by these last two preparations, the second representing a 1,600-fold purification of cholinesterase relative to the first, shows that the hydrolysis of murexine by human plasma is brought about by the cholinesterase and is not contributed to significantly by other plasma esterases. This conclusion is confirmed by the complete inhibition of hydrolysis obtained with 10 μM. eserine, an inhibitor and concentration specific for cholinesterases.

Animal Experiments

The effect of murexine on the respiration and blood pressure in the cat and dog and the effect on neuromuscular transmission in the dog are summarized in Table II.

Effect on Respiration.—In the dog, murexine at a dose level of 30 μg./kg. produced a transient increase in respiration. Increasing doses up to and including 500 μg./kg. caused a progressively longer and more pronounced increase. At 1 mg./kg. murexine produced a short

TABLE II

EFFECT OF MUREXINE ON RESPIRATION, BLOOD PRESSURE AND NEUROMUSCULAR TRANSMISSION IN THE CAT AND DOG

In column (f) the numerals show the change in twitch height with the range in brackets, + denotes an increase and — a neuromuscular block.

| Dose (μg./kg.) (a)	Respiration		Blood Pressure		Neuromuscular Transmission (% Change in Twitch Height), Dog (f)
	Cat (b)	Dog (c)	Cat (d)	Dog (e)	
10	—	—	None	None	—
30	None	Transient increase	—	—	—
50	None	⎫	None	Slight fall	None
100	None	⎬	None	Fall followed by slight rise	+9 (0 to +14)
200	Slight increase	⎪	Slight fall	⎫	+16 (+9 to +22)
300	—	Progressive increase	Slight rise	⎬	−40 (−14 to −60)
500	Stimulation followed by failure	⎭	Definite rise	Progressive rise	−80 (−52 to −88)
1,000	—	Stimulation followed by failure	—	Marked rise	−98 (−87 to −100)
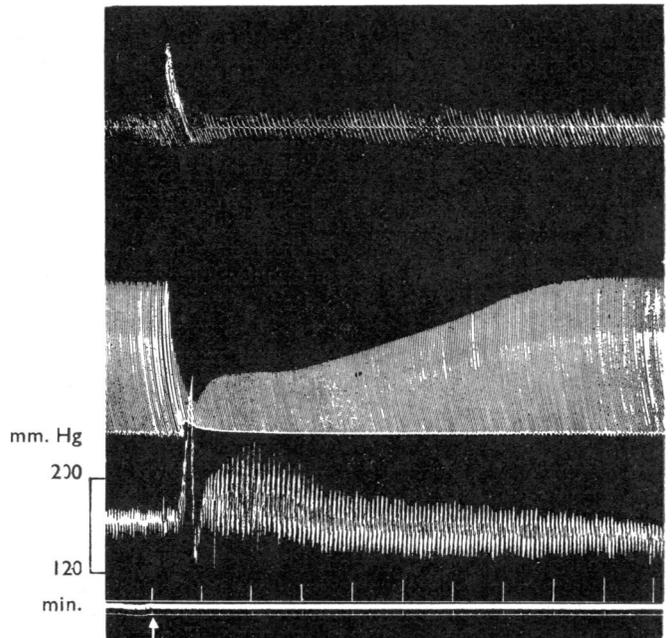
FIG. 1.—Effect of 0·55 mg./kg. of murexine injected intravenously at the arrow into the anaesthetized dog. Upper trace, respiration; middle trace, record of the isotonic twitch of the sciatic gastrocnemius muscle in the response to nerve stimulation; lower record, blood pressure.

mm. Hg
200
120
min.

stimulation followed by respiratory failure. Artificial respiration for 10 to 20 min. was always sufficient to save the animal. Respiratory failure was always manifest when the neuromuscular transmission, as indicated by the response of the sciatic-gastrocnemius preparation, was completely depressed, and was thus probably due to neuromuscular blockade in the respiratory muscles. Murexine in the cat produced the same effects, but a dose about seven times greater was needed to evoke effects than in the dog.

Blood Pressure.—A dose of 50 μg./kg. of murexine in the dog caused a slight depressor response. Increasing the dose up to 500 μg./kg. usually caused a depressor response followed by a pressor response of progressively greater amplitude and duration. The same effects were noted in the cat, but again the doses required were higher.

Neuromuscular Transmission. — Murexine caused no change in neuromuscular transmission in the dog in doses up to 100 μg./kg., while doses of 100 to 200 μg./kg. caused either no change or a slight increase in the twitch height of up to 20%. This effect might be due to an anticholinesterase action of the drug (Foldes, Erdös, Baart and Shanor, 1957). Above 200 μg./kg. blockade began to be manifest; this progressively increased with increasing doses and was 90 to 100% complete at 1 mg./kg. The type of neuromuscular blockade obtained with 550 μg./kg. of murexine in the dog is shown in Fig. 1, where the effects on respiration and blood pressure can also be seen.

The dose of murexine required to produce 90 to 100% neuromuscular blockade in three different mammalian species is presented graphically in Fig. 2. The corresponding doses of decamethonium are included for comparison. It will be seen that the species variations in the potency of the two drugs run parallel, the potency decreasing from the cat, through the dog, to the rat.

Effect of Tetraethylammonium (TEA) and Neostigmine.—TEA in a dose of 10 mg./kg. blocked the effect of murexine (500 μg./kg.) on blood pressure and neuromuscular transmission. The neuromuscular blocking action of murexine was not reversed by neostigmine in the dog or rat; indeed, in the rat, recovery was delayed by neostigmine.

Depolarizing Action.—It is well known that many neuromuscular blocking agents such as decamethonium and suxamethonium, exert their effect mainly by producing a prolonged depolarization of the endplate region. This is in contrast
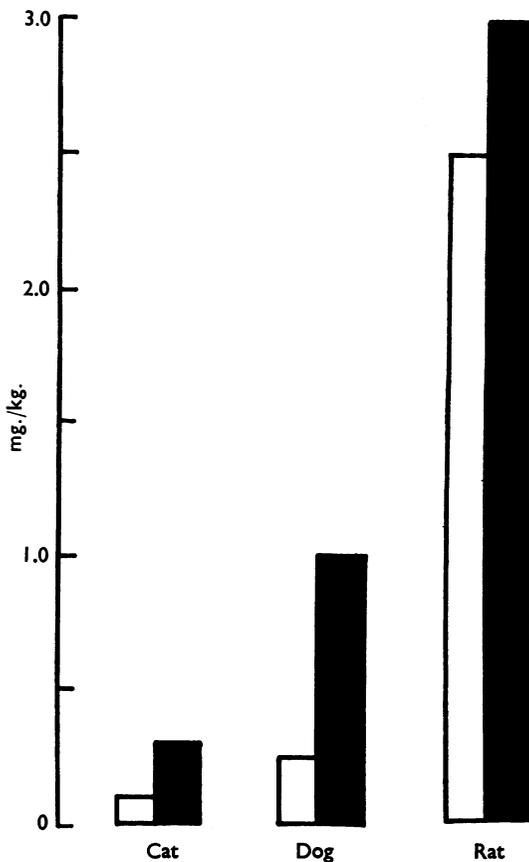

FIG. 2.—Species variation in the neuromuscular blocking action of murexine (solid columns) compared with that of decamethonium (open columns). Ordinates, dose of blocking agent required to produce 90 to 100% neuromuscular block.

to the type of blockade seen with tubocurarine, in which the drug blocks the depolarizing effect of acetylcholine without itself producing depolarization. It was of interest to find out if murexine had a direct depolarizing action on the endplate region, using the scanning electrode technique of Burns and Paton (1951). The arrangement of the electrodes and the potentials recorded are indicated in Fig. 3*a*. Fig. 3*b* shows that 40 sec. after the injection of the drug, the surface of the muscle in the endplate region became depolarized, as evinced by the negative potential recorded between a scanning electrode (*Sc*) and the stationary electrode (*St*) at the end of the muscle. Fig. 3*c* shows that the drug reduces the magnitude of the summed action potentials recorded by the scanning electrodes in response to periodic supramaximal stimulation of the motor nerve, showing that fewer
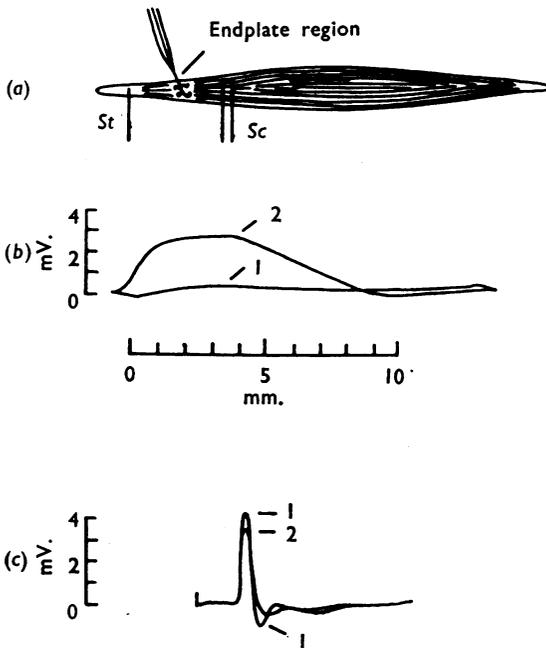
FIG. 3.—Depolarizing action of 3 mg./kg. murexine on endplate region of the gracilis muscle of the rat. (*a*) Diagram of electrodes used and potentials recorded. *St* is the stationary electrode and *Sc* the scanning electrodes. (*b*) Tracing of the potential of the scanning electrodes (*Sc*) relative to the stationary electrode (*St*) on the musculotendinous junction. Abscissa, distance of *Sc* from *St* in mm. (*c*) Tracings of the summed action potentials of the gracilis muscle under control conditions (1) and 40 sec. after injection of murexine (2). Negative potentials are shown as an upward deflexion of the traces. The potentials were recorded between the two scanning electrodes (*Sc*).

muscle fibres are responding to the nerve stimulation as the result of the blockade set up by the drug.

DISCUSSION

The results summarized above are in general agreement with those of other workers and show that murexine has both ganglion stimulating and neuromuscular blocking actions. The rise in blood pressure is most likely due to stimulation of sympathetic ganglia, since it was antagonized by the ganglion blocking agent TEA. Murexine is primarily a depolarizing type of neuromuscular blocking agent. This is shown by the spastic paralysis which it produces in birds (Erspamer and Glässer, 1957) in contrast to the flaccid paralysis produced by tubocurarine, the species variation in sensitivity to the drug, which closely parallels that of decamethonium, the inability of eserine to reverse the neuromuscular blocking

action, and, finally, its depolarizing action on mammalian endplates as demonstrated by the scanning electrode experiments. The combination of ganglion stimulating and neuromuscular blocking action is also seen in other compounds, such as tetrakis (dimethylaminomethyl)methane tetramethobromide (Kensler, Langemann and Zirkle, 1954), and, interestingly enough, in β,β-dimethylacryloylcholine (Holmstedt and Whittaker, unpublished observation), the choline ester which occurs in place of murexine in the Muricidae *Thais floridana* (Whittaker, 1957; Keyl *et al.*, 1957).

The action of murexine was short-lasting in all three species examined. This cannot be attributed to hydrolysis by plasma esterases, since in two of these species, rat and dog, the rate of hydrolysis of the ester by plasma was negligible (Table I). In the analogous case of suxamethonium, where hydrolysis by plasma cholinesterase has been advanced as explanation for the short-lasting action of the drug, there is also no correlation between the rate of plasma hydrolysis and the duration of neuromuscular blockade in different species (Hansson, 1958).

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