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Some actions of 4,17a-dimethyl-4,17a-diaza-D-homo-5α-androstane dimethiodide (HS-342), a new neuromuscular blocking drug†

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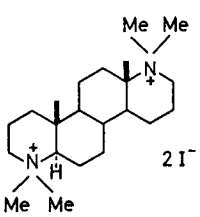
The effects of the newly-synthesized bisquaternary steroid 4,17a-dimethyl-4,17a-diaza-D-homo-5α-androstane dimethiodide (HS-342) have been investigated on the rat isolated phrenic nerve-hemidiaphragm, chick biventer cervicis and intact cat tibialis anterior muscle preparations, on the guinea-pig isolated ileum and on the rat isolated vas deferens preparations. The anticholinesterase activity of HS-342 was also measured. In the skeletal muscle preparations HS-342 exhibited properties of a post-junctionally active neuromuscular blocking agent of the non-depolarizing class. In the anaesthetized cat HS-342 exhibited approximately equal activity to that of tubocurarine, but its duration of action was about one-third of that of tubocurarine. HS-342 exhibited low anticholinesterase activity, low muscarinic blocking activity and apparently had no effect on the adrenergic neuroeffector junction studied.

Since the discovery of the neuromuscular blocking activity of the steroidal bisquaternary ammonium alkaloid malouétine (Quévauviller & Lainé, 1960), the steroid nucleus has been widely used as an almost rigid supporting moiety for quaternary ammonium groups, usually as α or β substituents on the 2, 3, 16 and 17 positions. Most of these compounds, particularly the bisquaternary compounds with interonium distances in the region of 10Å, possess neuromuscular blocking activity (see Martin-Smith, 1971 and Buckett, 1972 for reviews). Recently, compounds with interonium distances in the region of 6·5Å have been shown to possess predominantly ganglion blocking action (Marshall & Martin-Smith, 1972).

The subject of this paper is a chemically novel compound, 4,17a-dimethyl-4,17a-diaza-D-homo-5α-androstane dimethiodide (HS-342; I), in which the two quaternary ammonium groups are fused into the steroid nucleus. The compound has an interonium distance of approximately 8Å as measured from Dreiding models and was synthesized (Singh, Paul & Parashar, 1972; 1973) as a potential neuromuscular blocking drug.



(I)

† Steroids and Related Studies. Part XXII.

Some of these results were communicated to the British Pharmaceutical Conference at Keele in September 1972.

METHODS

Rat hemidiaphragm-phrenic nerve preparations

The rat phrenic nerve-hemidiaphragm preparation (Bülbring, 1946) was mounted in Krebs-Henseleit (1932) solution at 32° and bubbled with oxygen containing 5% carbon dioxide. The phrenic nerve was stimulated electrically using supramaximal rectangular pulses of 0·2 ms duration, at a frequency of 0·1 Hz. Isometric tension changes were recorded by a Grass FT03C force-displacement transducer.

Chick biventer cervicis nerve-muscle preparation

The chick biventer cervicis muscle preparation (Ginsborg & Warriner, 1960) was mounted and stimulated using the same conditions and parameters as used for the rat hemidiaphragm preparation.

Cat tibialis anterior—sciatic nerve preparation

The potency and duration of action of HS-342 were assessed on the tibialis anterior muscle—sciatic nerve preparation of the cats anaesthetized with a mixture of α-chloralose (8 ml kg^{-1} of a 1% solution) and pentobarbitone sodium (2·5 mg kg^{-1}) given intravenously. Isometric tension changes of the muscles were recorded by a Grass FT1OC force-displacement transducer. The stimulation parameters were the same as those used for the rat and chick muscle preparations.

Guinea-pig ileum preparation

Segments of guinea-pig ileum were mounted in Krebs-Henseleit solution at 37° and bubbled with oxygen containing 5% carbon dioxide. Isotonic contractions in response to agonists were recorded on smoked paper using a frontal writing lever loaded with a 1g mass and a magnification factor and time cycle.

Rat vas deferens preparation

The isolated vas deferens of the rat was mounted in Krebs-Henseleit solution containing atropine ($1{\cdot}44 \times 10^{-6}$M) at 32° and bubbled with oxygen containing 5% carbon dioxide. The post-ganglionic sympathetic nerves were stimulated with ring electrodes by 10 s trains of pulses (1 ms duration, 20 Hz frequency) at intervals of 10 min. Between each train of pulses noradrenaline (6×10^{-6}M) was added to the bath and allowed to remain in contact with the tissue for 60 s.

Anticholinesterase activity

The anticholinesterase activity of HS-342 was assessed by measuring the cholinesterase activity of homogenates of cat tibialis anterior and soleus muscle in the presence of the drug, using the colorimetric method of Ellman, Courtney & others (1961). Homogenates of cat tibialis anterior and soleus muscles were prepared using 1 g of

tissue in 20 ml of 0·1M phosphate buffer, pH 8·0. The homogenate (0·2 ml) was added to a spectrophotometer cell containing the inhibitor dissolved in phosphate buffer (2·8 ml) 0·1M pH 8·0, and also containing 5,5-dithiobis-2-nitrobenzoic acid (0·1 ml) 0·01M; 0·1 ml of the substrate acetylthiocholine (0·015M) was added to the cell and the absorbance change was measured at a wavelength 412 nm and temperature 25° using an SP800 spectrophotometer. The blank contained all the reagents except the substrate. The absorbance change per min was compared with that from experiments performed in the absence of inhibitor, and the concentration of the compounds producing 50% inhibition of the enzyme was calculated.

Drugs and materials

The drugs and materials used were acetylcholine chloride, acetylthiocholine iodide, atropine sulphate, carbachol chloride, 5,5-dithiobis-2-nitrobenzoic acid, noradrenaline acid tartrate (British Drug Houses); neostigmine methylsulphate (Roche); (+)-tubocurarine chloride (Koch-Light).

RESULTS

Neuromuscular blocking activity

In the isolated phrenic nerve-diaphragm preparation of the rat HS-342 exhibited approximately half the molar potency of (+)-tubocurarine in depressing the indirectly elicited twitches of the preparation. As with (+)-tubocurarine, the neuromuscular block was readily reversed by the addition of neostigmine (3×10^{-6}M) to the organ bath in the continued presence of HS-342. The most striking difference between the actions of HS-342 and (+)-tubocurarine in the diaphragm was the rate of recovery of twitch height after washing the tissue. In the case of HS-342, one wash for 20 s was sufficient to ensure full recovery of twitch height within 5 min, whereas (+)-tubocurarine required several washes before a much slower recovery took place (Fig. 1).

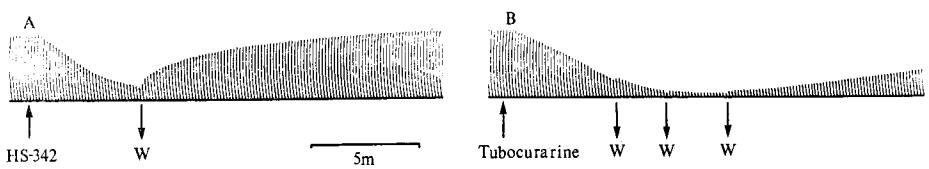


FIG. 1. Isolated rat phrenic nerve-hemidiaphragm preparation, stimulation frequency 0·1 Hz. A. HS-342 (2×10^{-6}M) was added and the tissue was washed by overflow for 20 s at W. B. (recorded 15 min after A) tubocurarine (1×10^{-6}M) was added and the tissue was washed three times before recovery of twitch tension commenced.

The phenomenon of rapid washout was also observed in the isolated chick biventer cervicis nerve muscle preparation, in which, on a molar basis, HS-342 was about 2·5 times more active than (+)-tubocurarine as a neuromuscular blocking drug. In this preparation HS-342 not only depressed twitch responses to nerve stimulation without itself causing contracture, but also depressed contractural responses to exogenous acetylcholine ($2{\cdot}14$–$3{\cdot}57 \times 10^{-4}$M) (Fig. 2a) indicating that the site of the blocking action is on the post-junctional acetylcholine receptors. The antagonistic action of HS-342 was studied in more detail in this preparation by constructing dose-response curves to carbachol in the presence of increasing concentrations of HS-342. HS-342 produced parallel shifts of the carbachol dose-response curve to the right without causing a reduction in the height of the maximal response attainable. Plots

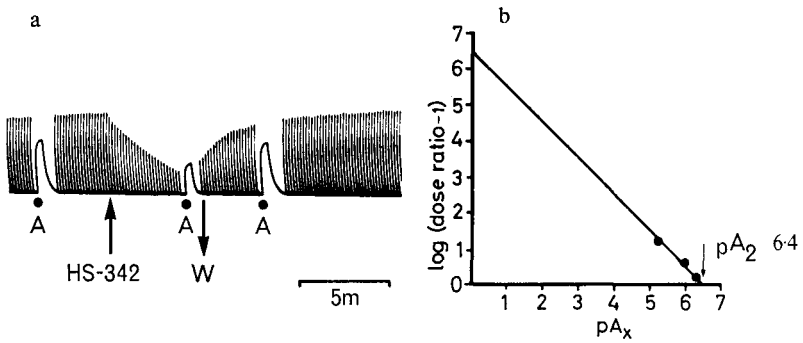


FIG. 2a Isolated chick biventer cervicis nerve-muscle preparation, stimulation frequency, 0·1 Hz. At A, acetylcholine (3×10^{-4}M) was added to the organ-bath for 30 s. HS-342 ($1{\cdot}5 \times 10^{-6}$M) reduced the responses to both nerve stimulation and exogenous acetylcholine. At W the bath was overflowed for 20 s.

b. Isolated chick biventer cervicis muscle preparation. Arunslakshana & Schild (1959) plots derived from dose-response curves to carbachol constructed in the presence of several concentrations of HS-342. The straight line plot of unit slope is indicative of competitive antagonism.

of log (dose ratio -1) against pA_x produced a straight line plot with a slope of unity (Fig. 2b), which is consistent with competitive antagonism (Arunslakshana & Schild, 1959). The calculated pA_2 value from extrapolation was 6·4.

Preliminary experiments in the anaesthetized cat showed that HS-342 was approximately equiactive with (+)-tubocurarine in depressing the twitches of the indirectly stimulated tibialis anterior muscle. The onset and duration of action were consistently shorter than those of (+)-tubocurarine, the onset being approximately twice as rapid, and the duration being about one-third in length.

Muscarinic blocking activity

HS-342 possessed weak antagonistic action against acetylcholine in the guinea-pig isolated ileum preparation. The block produced was competitive in nature, and the pA_2 value was calculated to be 5·15.

Actions on adrenergically-innervated tissue

In concentrations up to $1{\cdot}67 \times 10^{-5}$M HS-342 had no effect on the responses of the rat isolated vas deferens preparation to either transmural stimulation of the post ganglionic sympathetic nerves, or exogenous noradrenaline (6×10^{-6} M).

Anticholinesterase activity

HS-342 possessed an extremely weak inhibitory action against the cholinesterase in cat soleus and tibialis anterior muscle homogenates. Plots of percentage inhibition against concentration of HS-342 were constructed, and the PI50 values calculated from interpolation were $6{\cdot}5 \times 10^{-4}$M for soleus muscle homogenates and $1{\cdot}2 \times 10^{-3}$M for tibialis anterior muscle homogenates.

DISCUSSION

The results presented show that HS-342 possesses greater selectivity for blocking nicotinic receptors than for blocking muscarinic receptors compared with other standard blocking drugs. Thus the pA_2 value of 6·4 obtained for HS-342 on the

chick biventer cervicis muscle is close to the value of 6·0 obtained for (+)-tubocurarine on the same preparation (Gandiha, Green & Marshall, 1972). In contrast, the value of 5·15 obtained for HS-342 on the guinea-pig ileum preparation can be compared with that of 8·4 for atropine on this preparation (Schild, 1947). Thus HS-342 whilst being approximately equiactive with (+)-tubocurarine in blocking nicotinic receptors, is over 1000 times less active than atropine in blocking muscarinic receptors.

HS-342 showed only weak ability to inhibit the cholinesterase of cat skeletal muscle homogenates, on a molar basis being 5 to 10 times less active than is (+)-tubocurarine on such homogenates (Marshall, 1973). The low anticholinesterase activity of HS-342 is reflected in the ease of reversal of the neuromuscular block by neostigmine.

From the experiments on the rat vas deferens it also appears that HS-342 has a negligible effect on the adrenergic system, as α-adrenoceptor stimulation, adrenergic neuron blocking activity and inhibition of noradrenaline uptake would produce marked effects on the preparation.

The selectivity for nicotinic receptors was evidenced by HS-342 possessing a potent neuromuscular blocking action. The blocking action appeared to be purely of the non-depolarizing competitive type as HS-342 produced no contracture of the multiple-innervated chick biventer cervicis muscle, and the neuromuscular block was reversed by neostigmine in both chick and rat nerve-muscle preparations.

The ease of washout of HS-342 from isolated nerve-muscle preparations was particularly marked and may be related to the short duration of action of the compound in the anaesthetized cat, perhaps indicating that HS-342 is not as firmly bound to receptors or associated structures as is (+)-tubocurarine.

The onium centres of HS-342 are an integral part of the steroid nucleus in contrast to the bisquaternary steroids studied to-date, and therefore the high neuromuscular blocking activity of HS-342 is of interest. The structure of the compound probably allows the relatively flat steroid nucleus to approach the receptor surface more closely than do the previously studied compounds, and Van der Waal's forces may be important in binding the compound to the receptor surface, as they probably are in decamethonium (Lonsdale, Milledge & Pant, 1965) and tubocurarine derivatives (Marshall, Murray & others, 1967).

From studies on a wide range of steroidal and non-steroidal bisquaternary ammonium compounds it appears that the optimal separation distance of the onium heads for the production of neuromuscular blocking activity is in the region of 10–12Å (see Stenlake, 1963; Martin-Smith, 1971 for reviews), whereas Gill (1959) has proposed that, for ganglion-blocking activity, providing the cationic heads are small, 6–7·8Å is the optimal inter-onium distance. HS-342, with an inter-onium distance of about 8Å, falls between these optima and hence would be expected to possess both neuromuscular and ganglion-blocking activity. The results presented and further work in the cat (Marshall, Paul & Singh, 1973) show that this is so.

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