THE ANTIVERATRINIC ACTION OF SOME LOCAL ANAESTHETICS

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The activity of fifteen local anaesthetics against the "veratrine response" induced in the frog's sartorius muscle by 0·1 μg./ml. of veratridine was investigated. The suppression of potassium efflux by these agents, and the role played by this ion in the action of veratridine has also been examined.

Cocaine and procaine and its close analogues block nerve conduction without influencing membrane potentials (Bennett and Chinburg, 1946), whereas the blocking action of veratrine is accompanied by a depolarisation of the nerve membrane. Bishop (1932) and Shanes (1958) have, therefore, classified local anaesthetics and compounds with similar properties as membrane "stabilisers" in contrast to membrane "labilisers" like veratrine. It has also been reported that local anaesthetics stabilise the normal membrane potential of the nerve to depolarisation by veratrine. The action of veratrine and membrane stabilisers on nerves has been further studied by Herr and Akcasu (1960).

Since the *ortho*-substituted benzoic acid esters of dialkylaminoalkanols which resemble procainamide exhibited antiveratrinic activity (Arora and Das, 1956), other local anaesthetic agents have been investigated for their activity against the veratrine-induced skeletal muscle response.

Experimental

The following drugs were investigated.

I 2-(Pyrrolidin-l-yl)propyl 2,6-dimethyl-4-propoxybenzoate hydrochloride.
II 2-Methyl-2-(pyrrolidin-1-yl)propyl 2,6-dimethyl-4-propoxybenzoate hydrochloride.
III 2-(Pyrrolidin-l-yl)ethyl *p*-propoxybenzoate hydrochloride.
IV 3-(2-Methylpiperidino)propyl *p*-butoxybenzoate.
V 3-(2-Methylpyrrolidin-1-yl)propyl *p*-cyclohex-2-enyloxybenzoate hydrochloride.
VI α-(Diethylaminomethyl)-*p*-methoxyphenethyl *p*-butoxybenzoate.
VII 2-Diethylaminoethyl 3,4,5-trimethoxybenzoate hydrochloride.
VIII αα-Di(*o*-methoxybenzyl)methylamine lactate.
IX Di-(*o*-methoxy-α-methylphenethyl)amine lactate.
X 2-Di(*o*-methoxy-α-methylphenethyl)aminoethanol hydrochloride.
XI *p*-Butoxy-β-piperidinopropiophenone hydrochloride.
XII 3-Dimethylamino-2-phenoxypropiophenone hydrochloride.
XIII 3-Dimethylamino-2-phenoxy-1-phenylpropanol hydrochloride
XIV 2-Diethylaminoethyl *p*-hexyloxybenzilate hydrochloride.
Lignocaine.

These drugs are white, crystalline, odourless powders, soluble in distilled water. Aqueous solutions were stored in a refrigerator, and not used after 7 days; 1 per cent solutions of these compounds had a pH value ranging from 6 to 7·5, except those of XIII and VII, which had a pH value of 4. Quinidine was used as a standard drug for comparison.

Methods

Male frogs (*Rana tigrina*) weighing between 40 and 60 g. were used. Both the sartorius muscles were removed, suspended in twin chambers and attached to tension levers.

The fluid in which the muscles were placed contained: sodium chloride, 0·5; potassium chloride, 0·014; calcium chloride anhydrous, 0·011 and sodium bicarbonate, 0·23 per cent. The bathing fluid was gassed with 95 per cent oxygen and 5 per cent carbon dioxide throughout the experiment. Electrical stimuli were applied to the muscle in the bath by a Grass Stimulator (Model 34B). One electrode was attached to the clip holding the upper end of the muscle and the other to the silver outlet of the chamber. A small length of the muscle (about 5 mm.) was kept above the surface of the bathing fluid to ensure the completion of the circuit through the muscle at each stimulus. The strength of the stimulus was 40 V, its duration 0·5 msec. and its frequency every 2 min.

Prevention of the veratrine response. After keeping the sartorius muscles in the bicarbonate buffer for about 15 min., the stimulus was applied seven times at about 2 min. intervals. The bicarbonate solution containing a suitable concentration of the test drug was now substituted in one of the chambers. Seven stimuli, at 2 min. intervals, were then applied to both muscles. After this, both muscles were subjected to a concentration of veratridine (0·1 μg./ml.). Stimuli were applied again to see whether the drug under study afforded protection against the development of a veratrine response.

Abolition of the veratrine response. After obtaining a few 'normal' twitches of the sartorius muscles, veratridine was added to the bath fluid to give a concentration of 0·1 μg./ml. The veratrine response was allowed to develop fully in the two sartorius muscles. The test substance in a concentrated solution was then added to one muscle to give the desired concentration in the bicarbonate buffer. The muscle treated with veratridine alone served as control. After the test substance had abolished the fully developed veratrine response, the treated muscle was washed thoroughly with bicarbonate buffer solution, followed by the addition of veratridine, 0·1 μg./ml. The development of a typical veratrine response indicated that the effect was reversible.

Determination of K^+ efflux in the veratrinised sartorius muscle. The muscles were suspended in the usual manner in the twin chamber and left in the bath for 5 min., after which the fluid was withdrawn and preserved, to act as a "control" for the K^+ content every withdrawal was followed by the addition of fresh Ringer's solution. Fresh Ringer's fluid was added,

a single stimulus was applied, and, during the contraction, the fluid was withdrawn. After 5 min. 14 stimuli were applied in succession and the fluid was again withdrawn. The veratrine response was then developed with 0·1 μg./ml. veratridine. Sufficient time was allowed for the veratrine response to develop to its maximum, then a single stimulus and multiple stimuli were applied. Fluid was withdrawn during the contraction after a single stimulus and again after repeated stimuli. Every withdrawal was followed by the addition of fresh Ringer's solution. The antiveratrinic drug was then added, and when the veratrine response was abolished, the process of fluid withdrawal after a single stimulus, and after successive stimuli was repeated. The potassium content was determined spectrophotometrically.

Results

The concentrations of each drug were 10 μg./ml.; 3 μg./ml.; 1 μg./ml. and 0·3 μg./ml. Three experiments were made with each drug to observe the preventive action, and another 3 to test the abolition of the veratrine response. The evidence is given in Table I.

TABLE I

THE RELATION BETWEEN THE CONCENTRATION WHICH PREVENTED THE VERATRINE RESPONSE AND THAT WHICH ABOLISHED THE VERATRINE RESPONSE IN THE FROG SARTORIUS MUSCLE

Compound No.	Prevention of veratrine response		Abolition of veratrine response		
	No. positive experiments	Conc. (μg./ml.) which prevents	No. positive experiments	Conc. (μg./ml.) which abolishes	Average time min.
I	1 out of 3 3 out of 3	0·33 1·0	1 out of 3 3 out of 3	0·33 1·0	26 8
II	3 out of 3	3·3	3 out of 3	3·3	20
III	3 out of 3	0·33	3 out of 3	0·33	6
IV	2 out of 3 3 out of 3	0·33 1·0	1 out of 3 3 out of 3	0·33 1·0	26 6
V	1 out of 3 3 out of 3	0·33 1·0	1 out of 3 3 out of 3	0·33 1·0	30 4
VI	3 out of 3	3·3	3 out of 3	3·3	12
VII	3 out of 3	10·0	3 out of 3	10·0	12
VIII	2 out of 3 3 out of 3	0·33 1·0	1 out of 3 3 out of 3	0·33 1·0	18 8
IX	3 out of 3	1·0	3 out of 3	1·0	30
X	3 out of 3	3·3	3 out of 3	3·3	10
XI	3 out of 3	1·0	3 out of 3	1·0	10
XII	3 out of 3	1·0	3 out of 3	3·3	12
XIII	3 out of 3	3·3	3 out of 3	3·3	18
XIV	3 out of 3	3·3	3 out of 3	3·3	20
Lignocaine	0 out of 3 3 out of 3	3·3 10·0	0 out of 3 3 out of 3	3·3 10·0	— 2
Quinidine	2 out of 3 3 out of 3	3·3 10·0	1 out of 3 3 out of 3	3·3 10·0	26 12

Prevention of veratrine response. Compounds I, III, IV, V, VIII, IX, XI and XII exhibited strong antiveratrinic activity antagonising the veratrine response in concentrations of 0·3 and 1 μg./ml. Compounds II, VI, X, XII and XIV were effective in 10 μg./ml. concentrations. Compound VII and lignocaine were effective in 10 μg./ml. concentrations. Quinidine exhibited a greater antiveratrinic activity than VII and lignocaine, but was less potent than the rest of the drugs tested. Fig. 1 shows the prevention of the veratrine response with I in a concentration of 1 μg./ml.

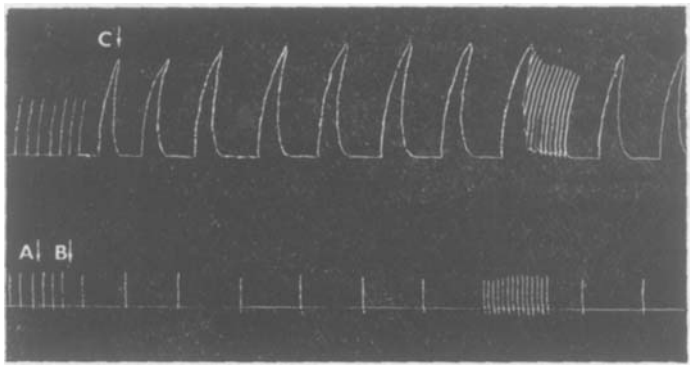

FIG. 1. Prevention of veratrine response by compound I. At Ag lower muscle was exposed to compound I (10^{-6}). After 7 stimuli at 2 min. intervals the upper muscle was treated with veratridine (10^{-7}) at C.

At B compound I was replaced by another solution containing compound I+ veratridine (10^{-7}).

Abolition of veratrine response. The drugs which were found to be more active in preventing the veratrinic response were also more potent in abolishing the response itself. However, in general it may be said that a higher concentration is necessary to bring about an abolition of the fully developed response. The effects were reversible as indicated by the re-establishment of the veratrine response.

TABLE II

POTASSIUM EFFLUX IN THE VERATRINISED SKELETAL MUSCLE OF FROGS

	K^+ efflux in m-equiv./litre in sartorious muscle Mean ± Standard deviation Range			Significance of K^+ increase after veratridine	Significance of K^+ decrease after drugs
	Control	After veratridine	After drugs		
Single stimulus	0·028 ± 0·006 0·012 – 0·048	0·031 ± 0·010 0·014 – 0·055	0·021 ± 0·007 0·011 – 0·036	$P < 0.05$	$P < 0.001$
After 14 stimuli	0·065 ± 0·008 0·05 – 0·09	0·12 ± 0·01 0·1 – 0·17	0·060 ± 0·007 0·046 – 0·094	$P < 0.001$	$P < 0.001$

Probability was calculated by applying Student's '*t*' test.

The abolition of the fully developed veratrine response using veratridine 0·1 μg./ml. by compound V is shown in Fig. 2.

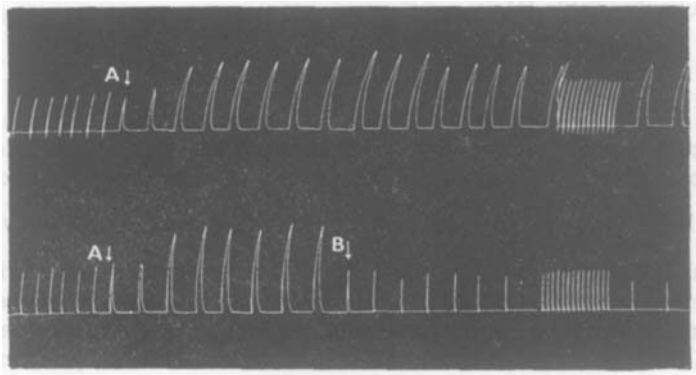

FIG. 2. (i) Abolition of the fully developed veratrine response.

(ii) The Veratrine response was allowed to develop fully in both muscles with veratridine (10^{-7}) added at A.

(iii) At B in the lower muscle a concentrated solution of compound V was added so as to make a desired concentration of the drug in the bicarbonate buffer solution. Soon after, the veratrine response was completely abolished.

Potassium efflux. In 35 experiments, the average potassium efflux during contraction of the sartorius muscle following a single stimulus and after applying 14 repeated stimuli was 0·028 and 0·065 m-equiv. per litre respectively. After the veratrine response was fully developed, following a single stimulus an average potassium efflux was 0·031 instead of 0·028 m-equiv., thereby showing that in the veratrinised muscle the efflux of potassium is increased. After bringing about abolition of the veratrine response with drugs, the diminution of potassium efflux was significant,

TABLE III

THE RELATION BETWEEN ANTIVERATRINE ACTIVITY AND LOCAL ANAESTHETIC POTENCY TESTED ON THE RABBIT CORNEA, AND RABBIT INTRADERMAL TEST

	Antiveratrinic activity			Local anaesthetic activity		
				MEC cornea conc. per cent	Intradermal	
Drug	Prevention	Abolition	Time in min.		Conc. per cent	Score
I	1×10^{-6}	1×10^{-6}	8	0·06	0·2	16
II	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	20	0·157	0·1	26
III	$3·3 \times 10^{-7}$	$3·3 \times 10^{-7}$	6	0·6	0·1	21
IV	1×10^{-6}	1×10^{-6}	6	0·06	0·05	9
V	1×10^{-6}	1×10^{-6}	4	0·03	0·25	22
VI	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	12	0·03	0·05	10
VII	1×10^{-5}	1×10^{-5}	12		10·0	20
VIII	1×10^{-6}	1×10^{-6}	8	0·01	0·01	19
IX	1×10^{-6}	1×10^{-6}	30	0·03	0·5	25
X	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	10	0·15	5·0	20
XI	1×10^{-6}	1×10^{-6}	10	0·05	0·4	12
XII	1×10^{-6}	$3·3 \times 10^{-6}$	12	0·3	0·5	20
XIII	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	18		0·5	12
XIV	$3·3 \times 10^{-6}$	$3·3 \times 10^{-6}$	20	1·5	0·4	22
Lignocaine	1×10^{-5}	1×10^{-5}	8	0·06	0·2	8

both after a single stimulus as well as after repeated stimuli. The average figures on addition of antiveratrinic drugs after single and repeated stimuli were 0·021 (before: 0·028) and 0·060 (before: 0·065) m-equiv./litre respectively (Table II).

Discussion

All the drugs investigated possessed both local anaesthetic and antiveratrinic activity. The rank correlation coefficient (R) was calculated between these two biological properties by applying the equation $R = 1-\frac{6\Sigma d^2}{n^3-n}$, where n is the number of drugs, d is difference between the ranks for the two different properties. For antiveratrinic activity, the concentrations for prevention and abolition of response were taken as a measure of potency; on the other hand the median effective concentration (MEC) and the intradermal anaesthetic score, determined by the methods elaborated by Chance and Lobstein (1944) and Bülbring and Wajda (1945) respectively, were taken as a suitable gauge for local anaesthetic activity. The actual values for the four said observations are given in Table III, which summarises the relative potencies of the various drugs. The rank correlation coefficient value on calculation was found to be 0·64.

Most of the strong antiveratrinic drugs were found to possess strong local anaesthetic activity. To cite a few examples, compounds I, III, IV, V, IX and XI were found to be potent in both respects.

The present investigation shows that the average potassium efflux during contraction of the frog's sartorius muscle following single and repeated stimuli was 0·028 and 0·065 m-equiv./litre respectively. After the development of the veratrine response the potassium efflux increased to 0·031 and 0·12 m-equiv./litre after single and repeated stimuli respectively. All the drugs exhibited an antiveratrinic activity and brought about a significant reduction in the potassium efflux both after a single stimulus and repeated stimuli, as is evident from Table II. The local anaesthetic action of drugs has also been partially explained by their ability to depress the potassium efflux from the nerve and thus bring about an interruption in nerve conduction.

Thus the potassium ion may be the common point for attack by the drugs for the biological activities, and the degree of impedence of potassium efflux through the plasmatic membrane may be responsible partly for the individual potency. In this study a parallelism between the two properties has been shown to exist, probably due to a common point of attack. This parallelism, however, is in no way fully explained, and demands a further solution.

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