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THE LOCAL ANAESTHETIC ACTIVITY OF A BENZOTRIAZINIUM SALT

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1 The local anaesthetic properties of 2-*n*-propyl-4-*p*-tolylamino-1,2,3-benzotriazinium iodide (TnPBI) were compared with those of lignocaine hydrochloride on intact and desheathed sciatic nerves of the frog, on the phrenic nerve-hemidiaphragm preparation of the rat, and by the intradermal wheal test in the guinea-pig.

2 Both TnPBI and lignocaine were more potent on desheathed than on intact sciatic nerves. The potency of TnPBI was affected more than that of lignocaine by the presence of the sheath in intact nerves.

3 Both drugs inhibited conduction in the rat phrenic nerve, as shown by the reduction in twitch tension of the diaphragm elicited by nerve stimulation. TnPBI also caused an initial augmentation of the twitch tension of the diaphragm when applied directly to the muscle.

4 TnPBI was shown to be approximately twice as potent as lignocaine by the guinea-pig intradermal wheal test.

5 These results are discussed in view of the known effects of TnPBI on intracellular calcium storage.

Introduction

A novel series of alkylated 1,2,3-benzotriazinium iodides was synthesized by Stevens & Stevens (1970). These compounds have a variety of actions on many isolated tissues, including the ability to cause contractures of the frog rectus abdominis and chick biventer cervicis muscles (Cull & Scott, 1973). These contractures are not mediated via cholinoceptors, but almost certainly result from an intracellular release of calcium ions from the sarcoplasmic reticulum and mitochondria (Muir & Scott, 1977). It was also noted by Cull (1972), that the benzotriaziniums possess local anaesthetic activity, and this paper compares the local anaesthetic properties of one of these compounds, 2-*n*-propyl-4-*p*-tolylamino-1,2,3-benzotriazinium iodide (TnPBI), with those of lignocaine.

Methods

Frog sciatic nerve

Sciatic nerves of *Rana temporaria* were used, either intact or desheathed. Stimulation of the nerves and recording of the compound action potentials were carried out by conventional techniques in a humidity cabinet. All experiments were performed at room temperature (18 to 22°C). The Ringer solution used had the following composition, (mM): NaCl 111, KCl 1.9, $CaCl_2$ 1.1, $NaHCO_3$ 2.4 and NaH_2PO_4 0.33. When gassed with air the Ringer had a final pH of 7.25 at 20°C. The nerves were stimulated antidromically by a Grass stimulator (model S48) through a Grass radio frequency stimulus isolation unit (model SIU5) at a frequency of 5 Hz, pulse width 0.1 ms and supramaximal voltage (50% above maximum). Action potentials were rendered monophasic, (by crushing the nerve between the recording electrodes) and displayed on a Tektronix dual beam oscilloscope (model 502A). Test solutions were added to a perspex cup, situated between stimulating and earth electrodes, volume 0.2 ml, through the top of which a 'V' shape notch was cut which enabled the nerve to pass through the meniscus of the solution. In intact nerves, the evoked action potentials were measured at 5 min intervals for 25 min and in desheathed nerves at 1 min intervals for 3 to 4 minutes. Control experiments were performed in which drug-free Ringer solution was applied to the nerve.

The ability of drugs to cause depolarization of desheathed nerves was measured (with minor modifications) by a sucrose gap technique developed by Stämpfli (1954). A three-chambered perspex bath was constructed with interconnections between the chambers, each of 2 ml volume, through which the nerve was passed and secured at each end. The inter-

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connecting holes were sealed with petroleum jelly and the chambers were then perfused with appropriate solutions by gravity inflow and suction outflow at 10 ml/minute. Depolarization was measured with Ag/AgCl electrodes embedded in agar-Ringer and encased in plastic 1 ml syringe barrels in contact with the fluid in the two outer chambers. The electrodes were connected via a Grass P16 DC preamplifier to a Washington Oscillograph (Model 400 MD2). Isotonic sucrose solution (246 mM) in deionised water was passed through an Elgastat B125 cartridge deioniser giving a solution with a specific resistance greater than 2 MΩ cm. This was perfused through the central chamber. Each preparation was left for 1 to 2 h to stabilize, after which test solution was perfused into one of the side chambers and any changes in electrical potential recorded. Control depolarizations were elicited by perfusion with 20 mM potassium chloride for periods of 2 min, while both lignocaine and TnPBI were perfused for periods of 15 minutes.

Rat phrenic nerve-hemidiaphragm preparation

Phrenic nerve-hemidiaphragm preparations were dissected out from adult albino rats of either sex and mounted in a tissue bath comprising four interconnecting chambers: the main chamber (10 ml) housed the muscle tissue while the phrenic nerve was carefully passed through a 1 mm hole in the side of the chamber and through a series of three chambers (each of 0.3 ml volume). The holes between all four chambers were then sealed with petroleum jelly. The muscle tissue was fixed on platinum hooks which also served as electrodes for direct muscle stimulation. The two outer chambers of the nerve bath each held a pair of 20 SWG platinum wire stimulating electrodes spaced 2 mm apart. The central nerve chamber was used to apply drug solutions to the nerve, while the two outer chambers were perfused with aerated Ringer solution. Thus the nerve could be stimulated at points proximal or distal to the drug solutions.

All four chambers were initially perfused (by gravity inflow and suction outflow) with a modified Krebs solution of the following composition (mM): NaCl 130, KCl 5.4, $CaCl_2$ 2.5, $MgSO_4$ 1.18, KH_2PO_4 1.1, $NaHCO_3$ 12.5 and glucose 11.1. When gassed with 95% O_2 and 5% CO_2, the solution had a pH of 7.20 at 20°C. All experiments were performed at room temperature (18 to 22°C) which prolonged the life of the tissue. Preparations were discarded in which the initial responses to distal and proximal nerve stimulation were different; shocks were delivered at supramaximal voltage, 0.2 ms duration and frequency 0.2 hertz. Control twitches of the diaphragm (measured with a Ugo Basile semi-isometric transducer and recorded on a Hewlett-Packard twin-channel recorder, model 7712) were obtained to distal stimulation for at least 30 min before drug solutions were introduced and, when reductions in twitch height occurred, brief periods of proximal stimulation were intermittently applied to ensure that the muscle was still able to produce a maximal response. In all experiments, the drugs were left in contact with the nerve until complete block was observed. The activity of the drugs was estimated by measuring the percentage reduction per minute of the magnitude of the twitch response.

Guinea-pig wheal

The technique of Bülbring & Wajda (1945) was used. Guinea-pigs of either sex, weighing 500 to 700 g were depilated 24 h before injection by clipping, shaving and then applying a depilatory cream. Four intradermal injections were made in each animal, two in the middle of the back and two over the hind quarter region, of low and high doses of lignocaine and of TnPBI. All possible combinations of drugs were injected in these areas. The applied stimulus to the injected areas was a pin prick delivered by a hand-held sharp needle. The scoring method was as described by the Edinburgh University Staff (1970). TnPBI was supplied by Dr M.F.G. Stevens of the University of Aston in Birmingham. Lignocaine hydrochloride was purchased from Willows Francis Ltd.

Results

Frog sciatic nerve

Local anaesthesia was measured as the mean percentage reduction per minute of the control action potential amplitude over 25 min drug contact. Table 1 compares lignocaine and TnPBI on both desheathed and intact sciatic nerves. In intact nerves, lignocaine and TnPBI had very similar rates of action at a concentration of 1×10^{-3} M (Figure 1). Recovery times, however, were very different; in the case of lignocaine (1×10^{-3} M), after complete block, 80% recovery occurred within 45 min of washing but after TnPBI (1×10^{-3} M) only 60% recovery was seen 3 h later.

In desheathed nerves both TnPBI and lignocaine were more potent than in intact nerves. The activity of both compounds was increased up to 10 fold, and recovery times were considerably shorter than in intact nerves (after lignocaine, recovery was almost complete within 10 to 15 min and after TnPBI 90% recovery occurred within 1 h of washing). Again similar rates of action were measured with the drugs at a concentration of 1×10^{-3} M.

Desheathed sciatic nerves mounted in the sucrose gap apparatus could be reversibly depolarized by the

Table 1 The effect of desheathing on the potency of lignocaine and TnPBI on frog sciatic nerve action potentials

Intact nerve			*Desheathed nerve*		
Drug	n	*% reduction per min*	*Drug*	n	*% reduction per min*
Control	12	0.1 ± 0.01	Control	7	1.7 ± 0.5
Lignocaine (M)			Lignocaine (M)		
1×10^{-3}	10	2.9 ± 0.1	2.5×10^{-4}	4	11.5 ± 1.5
2.5×10^{-3}	11	6.5 ± 0.3	5×10^{-4}	6	17.4 ± 1.6
5×10^{-3}	10	10.1 ± 0.5	1×10^{-3}	7	28.6 ± 1.7
TnPBI (M)			TnPBI (M)		
5×10^{-4}	8	2.5 ± 0.1	2.5×10^{-4}	6	15.2 ± 1.7
1×10^{-3}	15	2.7 ± 0.1	5×10^{-4}	6	20.7 ± 1.3
2.5×10^{-3}	8	3.5 ± 0.1	1×10^{-3}	7	25.1 ± 1.8

All values are the means of the number of experiments performed *(n)* together with the standard error of the means.

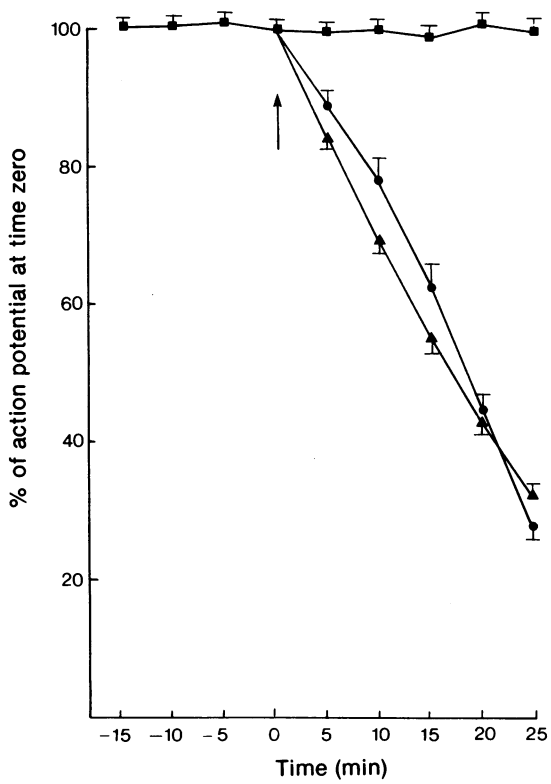

Figure 1 Comparison of the time course of the activities of 2-*n*-propyl-4-*p*-tolylamino-1,2,3-benzotriazinium iodide (▲) and lignocaine (●) on frog intact sciatic nerve; 1×10^{-3} M solutions of both drugs were applied at arrow; (■) control responses. Vertical bars represent s.e. means: $n = 12$ for control, 15 for TnPBI and 10 for lignocaine (separate nerves).

application of KCl (20 mM). Lignociane (1×10^{-3} M) caused little or no depolarization by itself, but reduced KCl-induced depolarizations by approximately 25%. TnPBI (1×10^{-3} M) produced a slight depolarization, and reduced the responses to KCl by about 75% (Table 2).

Rat phrenic nerve-hemidiaphragm

Both TnPBI and lignocaine applied to the phrenic nerve caused an irregular, 'stepped', progressive reduction of the muscle twitches to distal stimulation but did not affect the responses when the nerve was stimulated proximally, indicating nerve block between the electrodes. Table 3 summarizes their activity on this preparation.

Whenever TnPBI was added directly to the muscle bath an initial increase of about 5% in height of twitch responses was seen lasting for 5 to 10 min and followed by depression. Augmentation occurred either when the muscle was stimulated directly (in the presence of (+)-tubocurarine, 1×10^{-5} M, sufficient to abolish the responses to nerve stimulation) or when the phrenic nerve was stimulated. However, the subsequent depression of responses was smooth when the muscle was stimulated directly, in contrast to an irregular, stepwise pattern when the muscle was stimulated via the phrenic nerve. Both TnPBI and lignocaine were more potent in reducing twitch tension when added to the muscle bath compared with their application to the nerve; for example 5×10^{-5} M TnPBI in the muscle bath caused 6.7% reduction per min whilst 5×10^{-4} M in the nerve bath caused 4.7% reduction per minute. Lignocaine (1×10^{-4} M) added to the muscle bath caused 5.4% reduction per min whilst 1×10^{-3} M in the nerve bath caused 5.6% reduction per minute.

Table 2 The effect of TnPBI and lignocaine on potassium chloride-induced depolarization of frog desheathed sciatic nerve

	A (mV)	B (mV)	C (mV)	D (C/A × 100)
Lignocaine 1×10^{-3} M $n = 5$	17.72 ± 4.06	0.60 ± 0.24	12.76 ± 3.05	74.79 ± 8.7
TnPBI 1×10^{-3} M $n = 11$	14.16 ± 1.77	5.18 ± 0.63	3.56 ± 0.66	26.69 ± 4.03

All values are means of n observations ± s.e. mean.
A = pre-drug depolarizations to 20 mM KCl; B = drug-induced depolarization; C = post drug depolarization to 20 mM KCl; D = C values expressed as % of A values.

Guinea-pig wheal

The results from 8 guinea-pigs used in this experiment indicated that TnPBI was approximately twice as active as lignocaine. TnPBI, 0.83 and 2.5×10^{-3} M, caused 41 and 58% anaesthesia respectively, while lignocaine, 1.67 and 5.0×10^{-3} M, caused 39 and 62% anaesthesia respectively. The mean potency ratio was 1.88 (95% confidence limits, 1.33 to 2.59).

Discussion

TnPBI was shown to have considerable local anaesthetic activity on all three preparations used. Removal of the epineurium from frog sciatic nerve increased the potency of both TnPBI and lignocaine by a factor of about ten, indicating that the epineurium presents a considerable barrier to the penetration of both compounds. The effect of lignocaine is concentration-related on both intact and desheathed sciatic nerves whereas only after removal of the sheath was such a relationship apparent with TnPBI. It is possible that some lipid component of the epineurium impedes the penetration of TnPBI to the active nerve membrane and the time course of the recovery process further suggests this possibility; TnPBI-treated nerves required about four times as long to recover as the equivalent lignocaine-treated nerves. It is possible that a lipid component of the epineurium acts as a reservoir for TnPBI, which is highly lipid soluble (Cull & Scott, 1973), thus maintaining an effective blocking concentration round the active membrane.

The majority of local anaesthetics cause block of conducted impulses without a significant change in the membrane resting potential (Bennett & Chinburg, 1946). This was confirmed for lignocaine in the present study by its application to frog desheathed sciatic nerve using the sucrose gap technique. However, lignocaine did show some signs of antagonism towards KCl-induced depolarizations. TnPBI applied to the nerve did cause a slight depolarization and greatly reduced subsequent KCl-induced depolarizations. Fleckenstein, Hille & Adam (1951) observed that calcium, applied to the desheathed nerve, antagonizes KCl-induced depolarizations. It may be that TnPBI releases calcium from intracellular binding sites giving rise to a high local concentration adjacent

Table 3 Nerve conduction block by TnPBI and lignocaine in rat phrenic nerve-hemidiaphragm preparation

Drug	n	*% reduction of twitch response per min*
Lignocaine (M)		
2.5×10^{-4}	6	3.8 ± 0.2
5.0×10^{-4}	6	7.8 ± 0.3
1.0×10^{-3}	6	28.6 ± 3.8
TnPBI (M)		
2.5×10^{-4}	3	1.8 ± 0.2
5.0×10^{-4}	6	4.7 ± 0.5
1.0×10^{-3}	6	5.6 ± 0.4

All values are the means of the number of experiments performed *(n)* together with s.e. means.

to the axon membrane which then inhibits the effect of KCl. Of interest is the suggestion by Muir & Scott (1977) that TnPBI may cause contractures in frog skeletal muscle by a release of intracellular calcium.

The initial augmentation of muscle twitch size seen when TnPBI was directly applied to the diaphragm resembled the effect of quinidine on frog sartorius muscle (Lammers & Ritchie, 1955) and attributed by these authors to a prolongation of the time course of the active state of the muscle. TnPBI also increases the contraction time of the twitch fibres of the frog rectus abdominis muscle (C.K. Muir, personal communication). Augmentation may alternatively result from an increase in the duration of action of the released transmitter substance; Steinbach (1968), observing that lignocaine caused an increase in the duration of the endplate potential, suggested that this may be due to prolonged conductance changes caused by the transmitter in the presence of lignocaine. It appears that the augmentation observed on addition of TnPBI to the muscle chamber is due to an effect on the muscle itself or to an action on the prejunctional nerve terminals, since no such augmentation occurred when applied to the nerve.

The 'stepped' block of muscle twitch tension seen with TnPBI or lignocaine added to the phrenic nerve suggests the successive blockade of individual axons or axons of similar sensitivity to both agents. Since each nerve axon supplies a number of muscle fibres, failure of conduction in groups of axons could account for the irregular reduction in muscle twitch tension. A similar pattern of blockade was observed when either TnPBI or lignocaine was applied directly to the muscle bath; however, the concentrations of both drugs required to produce such blocks were approximately one-tenth of those required when applied to the phrenic nerve. Because of the aforementioned increase in potency on desheathed nerves, TnPBI and lignocaine when applied directly to the muscle bath might act on the fine nerve axons near the neuromuscular junction, which have neither epineurium nor insulating myelin (Galindo, 1971). If this were so then the 'stepped' block indicates a true local anaesthetic effect on the nerve axons rather than a postsynaptic effect. This suggestion is supported by the observation that when TnPBI was applied at higher concentrations to directly stimulated, curarized hemidiaphragm preparations, reduction of twitch tension was smooth and not stepped.

Further evidence of a local anaesthetic action of TnPBI comes from the experiments performed on the guinea-pig wheal preparation; although further work obviously needs to be done in this direction, the evidence suggests that TnPBI is approximately twice as potent as lignocaine in the intact animal.

It is well known that many substances with local anaesthetic activity have antiarrhythmic effects on cardiac muscle. Such effects of TnPBI are currently being investigated, and preliminary experiments indicate that the compound prolongs the action potentials of guinea-pig atrial and ventricle cells (French & Scott, 1977). This property is one which may be of value in the reversal of certain types of cardiac arrhythmia.

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