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THE EFFECT OF DDD ON SINGLE RANVIER NODES OF XENOPUS LAEVIS

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Membrane potentials of single Ranvier nodes of myelinated nerve fibres were measured. DDD suppressed the rising phase of the action potential, probably by a reduction of the inward sodium current. This effect is in sharp contrast to that of DDT, which does not affect the rising phase but slows down the falling phase of the action potential by prolonging the inward sodium current.

Myelinated nerve fibre
Single Ranvier node
DDD (2,2-bis(*p*-chlorophenyl)-1,1-dichloro-ethane)
Membrane potentials

1. INTRODUCTION

DDD is the product of reductive dechlorination of DDT. It is found as a metabolite of DDT in several species of animals and plants and as an impurity in technical DDT. It is also used as an insecticide (Rhothane). DDD is found in all biological samples containing DDT and is probably even more persistent than DDT itself (Perkow, 1968; O'Brien, 1967).

Although there are several publications concerning the toxicity of DDD (for references see Perkow and O'Brien), there is no information on its mode of action. It is now well established that DDT is a neurotoxicant and specifically affects the falling phase of the action potential in nerve fibres of lobster (Narahashi and Haas, 1968), frog (Hille, 1968) and toad (van den Bercken, 1968). There is only a small difference in molecular structure between DDT and DDD: DDT has three aliphatic-bound Cl-atoms, whereas DDD has two. The present investigation shows that this small difference in molecular structure causes a significant difference in effect on myelinated nerve fibres.

2. MATERIALS AND METHODS

The experiments were carried out on large (20–25 μ) single myelinated nerve fibres dissected from the sciatic nerve of the clawed toad (*Xenopus laevis*). The isolated fibre was mounted in a Perspex cell with three pools of Ringer's solution separated by two transverse partitions and sealed with silicone jelly (Verveen, 1965). The node under investigation was placed in the central pool, in which the normal Ringer's solution could be replaced by Ringer's solution containing DDD. Both side pools contained cocaine-Ringer's solution to make the neighbouring nodes unexcitable. The solutions in all three pools were repeatedly changed during the course of the experiments.

One set of Ag-AgCl electrodes was used to pass a current from one side pool through the isolated node in the central pool which was held at ground potential. The membrane potentials were recorded between the central pool and the other side pool by another set of Ag-AgCl electrodes connected to a negative capacitance electrometer amplifier. This side pool was

kept small to reduce the capacitance to earth. Electrical contact to the pools was made through Ringer-agar bridges.

DDD (gaschromatographically pure) was dissolved in ethanol. This solution was squirted rapidly through a no. 27 hypodermic needle into Ringer's solution giving a fine suspension. The final concentration of ethanol which was always less than 1% was found to have no appreciable effect in control experiments.

The composition of the Ringer's solution was (mM): NaCl 115, KCl 2.5, Na_2HPO_4 2.15, NaH_2PO_4 0.85 and $CaCl_2$ 2.0. All experiments were carried out at room temperature, 20–25°C.

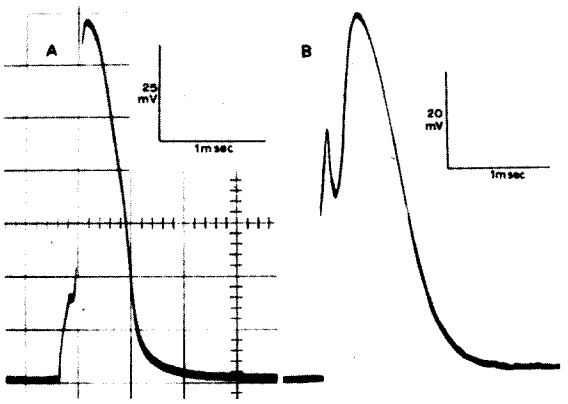

Fig. 1. (A) Action potential of an untreated node of Ranvier. (B) Action potential of the same node 2 min after the application of 5×10^{-4} M DDD. Attenuation is due to a change of solution in the central pool.

3. RESULTS

In an untreated node a stimulating current of about 1.5 nA for 100 μsec consistently elicited an action potential (fig. 1A).

The first effect of DDD (5×10^{-4} M) was to raise the threshold, slow the rate of rise and decrease the amplitude of the action potential; the falling phase remained almost normal (fig. 1B). There was also a prolongation of the refractory period. If in this stage of poisoning a slight hyperpolarization preceded the stimulus, a complete restoration of the action potential occurred.

Later there was a progressive increase in the threshold level, a decrease in the amplitude of the action potential and a prolongation of the falling phase (fig. 2A). After 15 to 30 min the membrane response to a short depolarizing current was nearly abolished. Washing with fresh Ringer's solution to remove DDD gave some improvement but complete recovery was not observed.

A noticeable change in resting potential (measured as the steady-state potential difference between the central pool and one side pool) was not observed in any of the experiments even those with high DDD concentrations (up to 10^{-3} M) and with long exposure (up to 2 hr).

Even in the last stages of poisoning a hyperpolarizing prepulse of sufficient amplitude brought the threshold level and the amplitude of the action potential back to nearly normal. The rate of rise could not be completely restored. The hyperpolarization had little, if any, restorative effect on the prolonged falling phase so that the duration of the restored action potential was about twice the duration of a normal action potential (fig. 2B). The effect of the hyperpolarization depended on the duration and amplitude of the prepulse and on the time delay between the prepulse and depolarizing pulse.

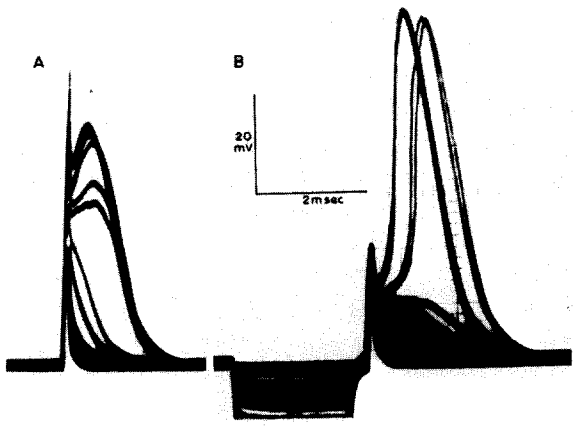

Fig. 2. (A) Nodal membrane responses to a short depolarizing current of increasing amplitude 10 min after the application of 5×10^{-4} M DDD.
(B) Restoration of the action potential by a hyperpolarizing prepulse of increasing amplitude.
In both photographs several traces are superimposed.

5. DISCUSSION

The rising phase of the action potential is controlled by a rapid increase in the sodium permeability of the nerve membrane, leading to an enhanced inward sodium current. The falling phase is regulated by a reduction in the permeability to sodium and an increase in the permeability to potassium, resulting into an increased outward potassium current (Dodge, 1963).

DDD probably reduces inward sodium current since its early effects on the rising phase of the action potential are identical to those of tetrodotoxin, xylocaine and prochlorperazine which specifically reduce the inward sodium current in frog's myelinated nerve fibres as revealed by voltage-clamp measurements (Hille, 1968).

The prolongation of the falling phase of the action potential in the later stages of poisoning might be explained by a delayed reduction in the permeability to sodium, by a suppression of the potassium current, or by both.

Comment can be made on the mechanism by which the hyperpolarizing prepulse restores the action potential although elucidation of the mechanism necessitates the use of the voltage-clamp technique.

The main effect of hyperpolarization is a removal of sodium inactivation; i.e. hyperpolarization decreases that portion of the sodium-carrying system which cannot undergo a rise in conductance when the membrane is depolarized (Dodge, 1963). The restoration of the action potential by a hyperpolarizing prepulse after DDD-treatment could thus indicate that DDD increases the amount of inactivation at the resting potential. According to Vallbo (1964) and Frankenhaeuser and Vallbo (1965) there is a close relationship between the critical slope of accommodation to linear rising currents and the steady-state inactivation of the sodium permeability. In two fibres the critical slope was measured just before and after the application of DDD but no large difference was found. This suggests that inactivation is not the major factor in reducing the inward sodium current.

There is a striking contrast between the early effect of DDD and the effect of DDT since DDT has no effect on the rising phase of the action potential in myelinated nerve fibres but slows down the rate of fall by a prolongation of the inward sodium current (Hille, 1968). Both agents affect the sodium channels of the nodal membrane but in a rather different way.

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