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THE ACTIONS OF PENTOBARBITONE, PROCAINE AND TETRODOTOXIN ON SYNAPTIC TRANSMISSION IN THE OLFACTORY CORTEX OF THE GUINEA-PIG

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1 It has been suggested that the depression of excitatory synaptic potentials produced by general anaesthetics can be attributed to a partial blockade of impulse conduction in the terminal branches of axons. This hypothesis has been tested by comparing the actions of pentobarbitone, procaine and tetrodotoxin (TTX) on synaptic transmission in the guinea-pig olfactory cortex.

2 Pentobarbitone (0.1–0.3 mM) depressed the evoked synaptic potentials without any significant depression of impulse conduction in the afferent fibres of the lateral olfactory tract (l.o.t.). It had no effect on the electrical excitability of either the l.o.t. axons or the postsynaptic neurones.

3 Tetrodotoxin (TTX; $1-5 \times 10^{-8}$ M) slowed conduction of impulses in the l.o.t. and decreased the amplitude of the l.o.t. compound action potential in proportion to the concentration applied. All concentrations of TTX elevated the electrical threshold of the l.o.t. axons and there was evidence to suggest that the threshold of the postsynaptic neurones was also elevated. The synaptic potentials were depressed in direct proportion to the depression of the l.o.t. compound action potential.

4 Procaine (0.1–0.5 mM) exhibited a pattern of activity intermediate between pentobarbitone and TTX. The most marked effect, seen at all concentrations tested, was a slowing of impulse conduction and a decrease in the electrical excitability of the l.o.t. axons.

5 It is concluded that general anaesthetics (exemplified by pentobarbitone) depress synaptic transmission by interfering with the processes involved in chemical transmission and not by blocking impulse conduction in the terminal branches of afferent nerves.

Introduction

Although there is abundant evidence that many general anaesthetics depress excitatory synaptic transmission within the CNS, there remains some uncertainty about the precise mechanisms involved. A number of studies have shown that, in peripheral nerves, myelinated fibres of small diameter are more readily blocked by local anaesthetics than those of large diameter (Gasser & Erlanger, 1929; Nathan & Sears, 1961). Furthermore, the amplitude of the excitatory postsynaptic potential of the giant synapse of the squid stellate ganglion is strongly dependent upon the amplitude of the action potential invading the presynaptic nerve terminal (Katz & Miledi, 1966). This had led to the suggestion that general anaesthetics may depress synaptic transmission by virtue of a partial blockade of impulse conduction in the small diameter terminal branches of afferent nerve fibres (Frank & Sanders, 1963; Seeman, 1972; Staiman & Seeman, 1974). This hypothesis is strengthened by the knowledge that local anaesthetics can be used to produce general anaesthesia (Frank & Sanders, 1963) and that general anaesthetics can block impulse conduction in nerve trunks (Seeman, 1972). This idea has obvious attractions since it implies that local and general anaesthetics have a common mechanism of action, the blockade of nerve impulse conduction. If true, it greatly simplifies the search for the molecular basis of anaesthetic action.

Although it is possible to marshal a number of indirect arguments against this view (see for example Somjen, 1963; Weakly, 1969; Richards, 1972), they have remained unconvincing in the absence of techniques for recording directly from the nerve terminals themselves. However, if impulse conduction in the nerve terminals is the primary target for the action of general anaesthetics at central synapses, it should be possible to mimic the selective depression of synaptic potentials that is observed with general anaesthetics such as the barbiturates with agents such as TTX that specifically and selectively block impulse conduction (Kao, 1966). This prediction has been tested by comparing the actions of pentobarbitone, procaine and TTX on synaptic transmission in *in vitro* slice

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preparations of the olfactory cortex of the guinea-pig. A preliminary communication of these results has been made to the Physiological Society (Richards, 1980).

Methods

Full details of the methods of preparation, incubation, stimulation and recording have been given elsewhere (Richards, 1981). Briefly, male guinea-pigs 250–400 g were stunned by a blow to the back of the neck, and the spinal cord severed before the brain was removed. Slices of olfactory cortex were cut with a razor strip and glass template. The slices so obtained had a nominal thickness of 420 μm and were incubated at 37°C in the chamber described by Richards & Tegg (1977), which permits a slice to be superfused by a stream of oxygenated artificial cerebrospinal fluid (c.s.f.) over both cut and pial surfaces. The slice was placed in the recording chamber immediately following its removal from the brain and recording started after a further period of 30–60 min.

Stimulation of the slices was effected by a pair of silver wires, insulated except at their tips, that were placed across the lateral olfactory tract (l.o.t.) at its anterior end close to the point of exit from the olfactory bulb. Stimuli were supramaximal except during tests of excitability and were delivered at 0.2 Hz. The evoked field potentials that followed each stimulus were recorded from the superficial layers of the cortex by means of glass micropipettes of approximately 2 μm tip diameter filled with 0.5 M NaCl. The potentials were recorded monopolarly, the indifferent electrode being a silver-silver chloride wire placed directly in the fluid of the recording chamber. Conventional methods of amplification and display were used.

The artificial c.s.f. used to bathe the preparations had the following composition (mM): NaCl 134, KCl 5.0, KH_2PO_4 1.25, $CaCl_2$ 1.0, $MgSO_4$ 2.0, $NaHCO_3$ 16 and glucose 10. It was saturated with 95% O_2, 5% CO_2 before use and had a pH of 7.3–7.4 at 37°C. The drugs were applied to the preparation in solution and did not significantly change the pH of the bathing medium.

The potentials elicited in the prepiriform cortex in response to stimulation of the l.o.t. have a characteristic appearance (see Yamamoto & McIlwain, 1966; Richards & Sercombe, 1968) and were measured as described by Richards, Russell & Smaje (1975) with the difference that, as the latency of onset of the synaptic potential (population e.p.s.p.) increased (with TTX and procaine), its amplitude was always measured at a fixed latency from its onset (usually 0.8–1.2 ms after onset).

Electron microscopy

This was kindly performed by Professor L.W. Duchen of the Institute of Neurology, London. Two guinea-pigs were anaesthetized with pentobarbitone and their brains were subsequently fixed with a solution of 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) by perfusion through a carotid artery. After fixation, the brains were removed and the olfactory cortex was dissected out and cut into small blocks together with the l.o.t. These blocks were then post-fixed in 2% OsO_4, embedded and sectioned. The resulting sections of the l.o.t. were viewed and randomly selected fields photographed in an electron microscope. The resulting plates were printed and the cross-sectional area of the l.o.t. axons estimated by means of a planimeter. From the estimated cross-sectional area, the equivalent diameter of each axon was calculated, assuming circularity.

Results

The properties of the lateral olfactory tract

The lateral olfactory tract of the guinea-pig is visible on the anterior ventrolateral surface of the brain as a narrow strip of white matter approximately 1 mm wide and 6–7 mm long. It consists chiefly of a bundle of thinly myelinated nerve fibres which are interspersed with glial cell processes and dendritic branches from the underlying nerve cells. It is similar in its essential features to that of the rat (Price & Sprich, 1975). The mean cross-sectional area of the axons (excluding the myelin sheath) is 1.13 μm^2 (range 0.07–6.78 μm^2, $n = 189$). The mean of the calculated equivalent diameters of the l.o.t. fibres is 1.23 ± 0.42 μm (mean ± s.d., $n = 189$). Along the macroscopically visible portion of the tract the conduction velocity is 8.32 ± 0.3 ms^{-1} (mean ± s.d., $n = 7$) but, as the fibres leave the main bundle and branch out over the surface of the cortex, the conduction velocity decreases rapidly with the distance from the tract (see Figure 1). The progressive decrease in conduction velocity as the action potential moves away from the l.o.t. presumably reflects a progressive reduction in the diameter of the tract fibres as they branch and distribute themselves over the surface of the cortex (see also Kerr & Dennis, 1972; Haberly, 1973). Associated with the decrease in conduction velocity is a decrease in the amplitude of the compound action potential.

For comparison, the mean cross-sectional area of the l.o.t. axons of the rat is 1.1 μm^2 at a level equivalent to that of the present study. This area corresponds to an equivalent diameter of 1.2 μm (Price & Sprich, 1975). The conduction velocity of rat l.o.t. fibres has

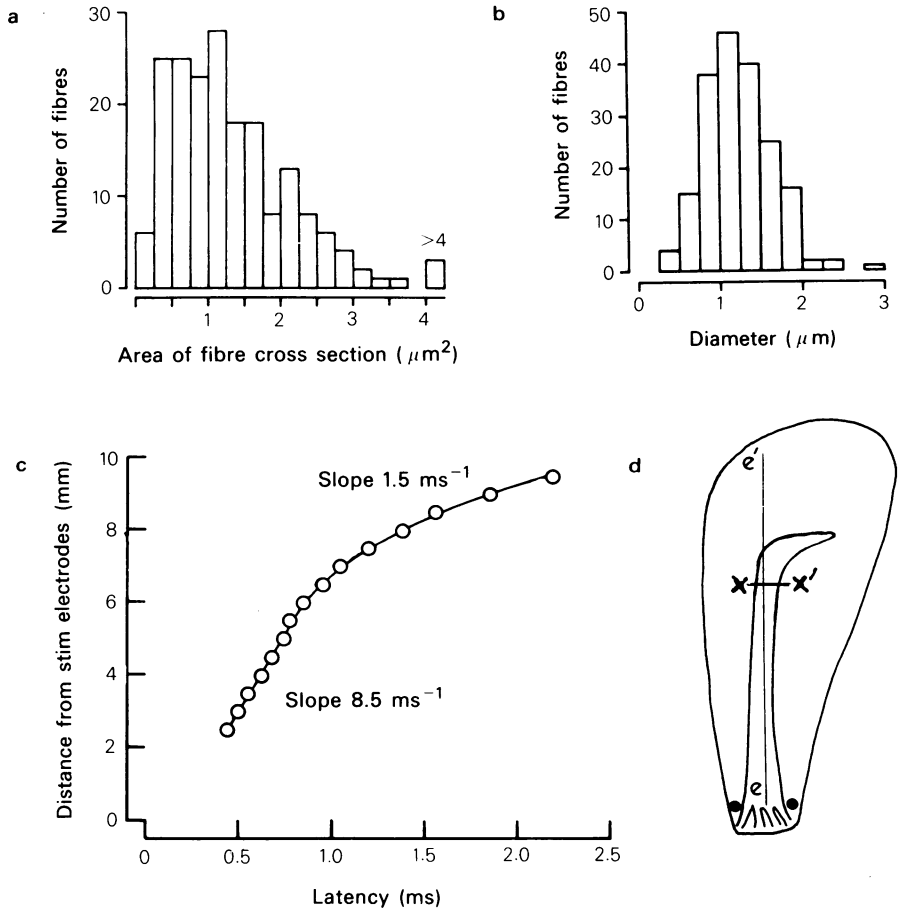

Figure 1 Fibre size and conduction velocity of the lateral olfactory tract (l.o.t.). (a) Histogram of cross sectional area of l.o.t. axons (excluding the myelin) at the level of the tract corresponding to X–X′. (b) Histogram of fibre diameters calculated from the cross-sectional area of l.o.t. axons assuming a circular profile. (c) Variation of conduction time with distance along the line e–e′ from the stimulating electrodes (●) as indicated in (d). The conduction latency was estimated as the difference between the onset of the stimulus artifact and the cross-over from the positive to the negative phase of the l.o.t. compound action potential.

been measured in slices of rat olfactory cortex as 7.03 ± 0.85 ms^{-1} (mean ± s.d., $n = 6$). The measured conduction velocity for the axons of the l.o.t. in both the rat and the guinea-pig agree well with that calculated from the 5.5–6 ms^{-1} μm^{-1} derived from measurements on peripheral nerves (Gasser & Grundfest, 1939; Waxman & Bennett, 1972).

Actions of tetrodotoxin and anaesthetics on the evoked potentials

If a recording electrode is placed on the surface of the prepiriform cortex close to the l.o.t. and a supramaximal stimulus is given to the anterior end of the l.o.t., a characteristic potential can be recorded which comprises an initial diphasic wave, the l.o.t. compound action potential, followed by a negative wave upon which may be superimposed one to three positive peaks. The negative wave has been identified as the field potential originating from the synchronous activation of many excitatory synapses (population e.p.s.p.) and the positive peaks represent the envelope of the discharge of the cortical cells in response to this excitation and are therefore called population spikes (see Richards & Sercombe, 1968; 1970).

When pentobarbitone (0.1–0.5 mM) was applied to an *in vitro* preparation of the olfactory cortex, the population e.p.s.p. and associated population spikes were both depressed without any concomitant change in the amplitude or time course of the compound action potential (see Figure 2 and Richards, 1972). This and other similar observations form the

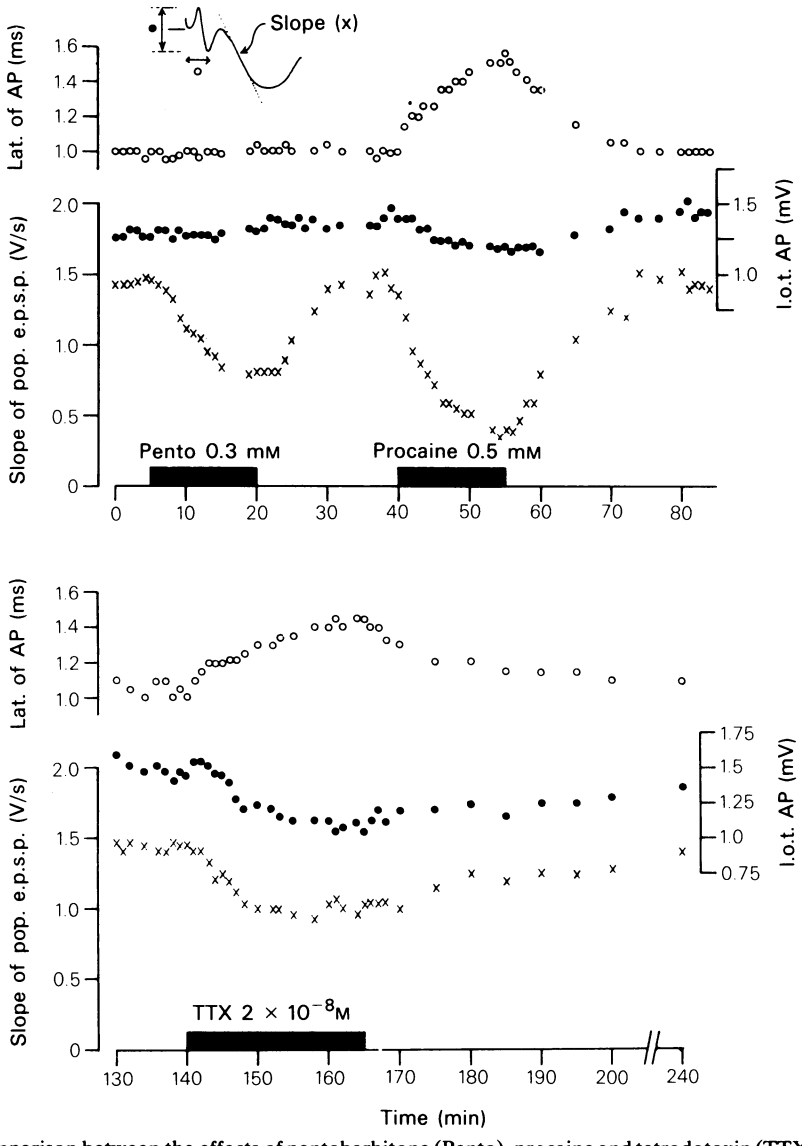

Figure 2 Comparison between the effects of pentobarbitone (Pento), procaine and tetrodotoxin (TTX) on impulse conduction of and synaptic transmission in a single *in vitro* preparation of the olfactory cortex.

basis of the idea that barbiturates (and other general anaesthetics) have a selective action on synaptic transmission (for review see Richards, 1978). However, when procaine (0.1–0.5 mM) or TTX ($1-5 \times 10^{-8}$ M) were applied to slices of olfactory cortex, the most obvious initial change was an increase in the time course and latency of the compound action potential. This was followed by a decrease in the amplitude of the action potential as the effect of these two drugs increased; the changes in the spikes and population e.p.s.p. being clearly secondary to the change in the amplitude of the compound action potential. That is, the decrease in the population e.p.s.p. paralleled the changes in the amplitude and latency of the compound action potential (see Figure 2).

A more detailed analysis of the actions of all three agents on impulse conduction in the l.o.t. showed that pentobarbitone had little or no effect on impulse conduction while procaine and TTX both caused significant dose-related decreases in conduction velocity. For example, 0.25 mM procaine caused a 14%

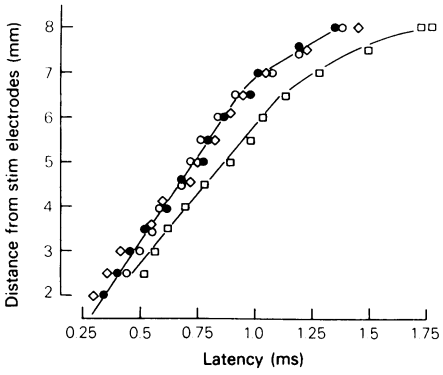

Figure 3 Action of pentobarbitone 0.3 mM (◇) and procaine 0.5 mM (□) on impulse conduction in the lateral olfactory tract. All the results are from a single preparation. Control (○); recovery (●).

and 0.5 mM a 25% decrease in conduction velocity in one preparation. In two further preparations 2×10^{-8} M TTX and 5×10^{-8} M TTX caused a decrease in conduction velocity of 28% and 55% respectively. A comparison of the actions of procaine and pentobarbitone on impulse conduction in the l.o.t. for a single preparation can be seen in Figure 3. Note that the region of slow conduction (corresponding to conduction in the branches of the l.o.t. axons) is not significantly affected by pentobarbitone. The effects of TTX were essentially similar to those of procaine (not shown).

Both procaine and TTX caused a striking increase in the electrical threshold of l.o.t. fibres whereas pentobarbitone did not change their excitability (compare Figure 4 of this paper with Figure 6 of Richards, 1972; data for procaine not shown). If the amplitude of the population e.p.s.p. is plotted as a function of the amplitude of the l.o.t. compound

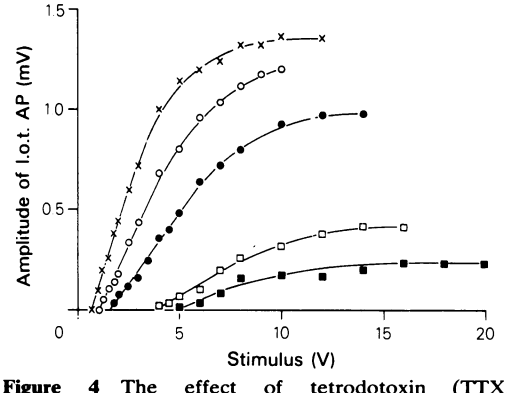

Figure 4 The effect of tetrodotoxin (TTX, $1-5 \times 10^{-8}$ M) on the electrical excitability of the lateral olfactory tract (l.o.t.). Control (×); tetrodotoxin 1×10^{-8} M (○), 2×10^{-8} M (●), 3×10^{-8} M (□), 4×10^{-8} M (■). Similar results were obtained with procaine 0.25–0.5 mM.

action potential elicited by stimuli of varying intensity, it can be shown that the slope of this function is reduced by pentobarbitone (0.1–0.5 mM) without changing the maximum amplitude of the l.o.t. compound action potential (see Figure 5). This shows that pentobarbitone has a selective depressant action on the e.p.s.p. In contrast, the same function derived with a preparation bathed in TTX ($1-5 \times 10^{-8}$ M) shows that the e.p.s.p. amplitude is reduced in direct proportion to the reduction in the amplitude of the l.o.t. compound action potential. The slope of the relation is not significantly altered but the maximum amplitude of the l.o.t. compound action potential and e.p.s.p. are reduced (Figure 5c). Procaine shows an intermediate behaviour. At low concentrations (0.25 mM) procaine has a non-selective action on the evoked potentials, the reduction in the e.p.s.p. paralleling the reduction in the compound action potential (Figure 5b). At higher concentrations (0.5 mM) the slope of the relation between l.o.t. action potential and the e.p.s.p. is also reduced (Figure 5d).

As I have previously reported (Richards, 1972) pentobarbitone did not change the relationship between the amplitude of the population e.p.s.p. and the area of the first population spike, the area of the population spike being an index of the number of neurones discharging in response to the synaptic excitation. However, TTX caused a rightward shift in this relationship in two out of three cases examined (Figure 6). This indicates that the depression of the population spike area by TTX was due in part to an increase in the electrical threshold of the postsynaptic cells. Procaine did not affect the e.p.s.p.-population spike relationship. The fact that procaine (and TTX in one experiment) did not alter the relationship between the e.p.s.p. and the population spike, despite the fact that both TTX and procaine clearly elevated the electrical threshold of the l.o.t. fibres, shows that the depression of postsynaptic cell discharge is chiefly due to the reduction in the excitatory synaptic drive to these cells and not to a decreased electrical excitability.

Discussion

The object of this study was to test the hypothesis that the depression of excitatory synaptic potentials by general anaesthetics can be attributed to a partial blockade of nerve impulse conduction in the fine terminal branches of afferent axons. The basis of this argument was the fact that both local and general anaesthetics can block nerve impulses and that the concentration of anaesthetic required for blockade decreases with decreasing fibre diameter (see Staiman & Seeman, 1974). As I have pointed out in the Introduction, if this hypothesis were true, it

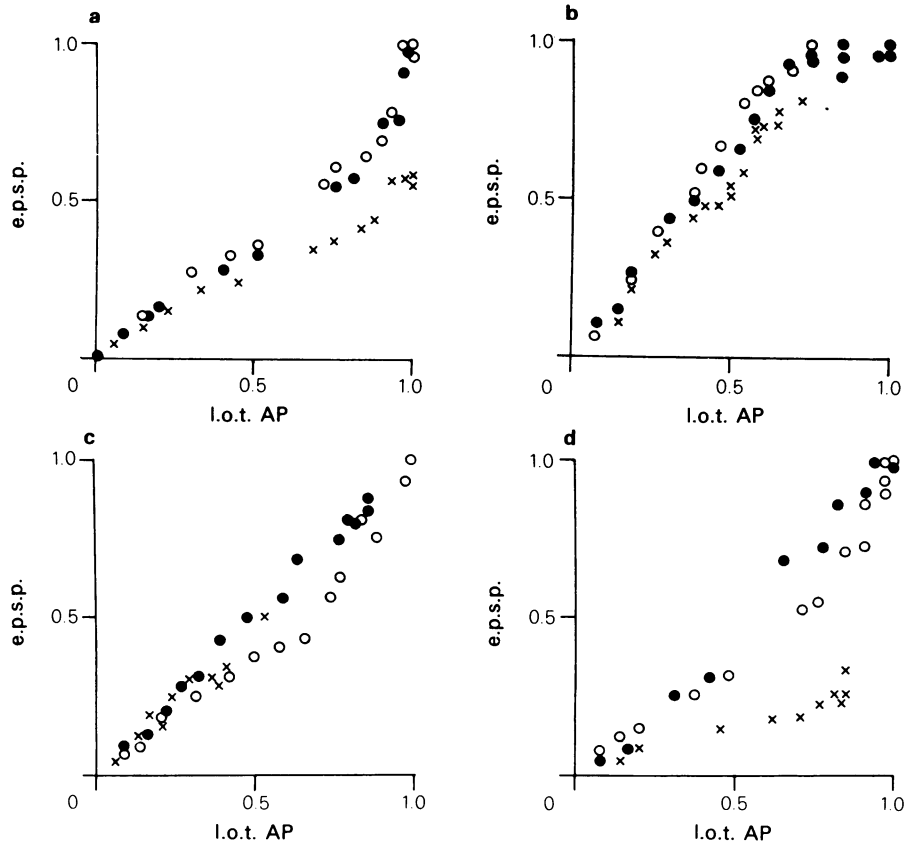

Figure 5 Effect of pentobarbitone, procaine and tetrodotoxin (TTX) on the relation between the amplitude of the lateral olfactory tract (l.o.t.) compound action potential (AP) and the population e.p.s.p. (a) Pentobarbitone 0.3 mM; (b) procaine 0.25 mM; (c) TTX 2×10^{-8} M; (d) procaine 0.5 mM. Control (○); drug treated (×); recovery (●). All values are expressed as a fraction of those obtained by maximal stimulation in the absence of drugs. Panels (a), (c) and (d) show results from the same preparation.

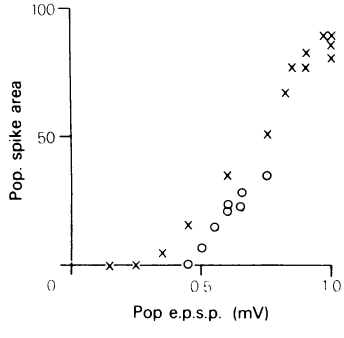

Figure 6 Relation between the area of the population spike and the amplitude of the population e.p.s.p. in the presence (○) and absence (×) of tetrodotoxin (TTX) 10^{-8} M. Note the rightward shift in the relation when TTX was applied. This indicates that TTX had increased the electrical threshold of the postsynaptic cells.

should be possible to mimic the action of a general anaesthetic with the puffer-fish toxin, tetrodotoxin, which is a specific blocking agent for the sodium channel of nerve and muscle (see Kao, 1966). The results show clearly that while pentobarbitone depressed the synaptic potentials of the olfactory cortex, it did so without significantly affecting the conduction of impulses by the l.o.t. This is in agreement with earlier results with this and other preparations (Larrabee & Posternak, 1952; Weakly, 1969; Richards, 1972). In contrast, the depression of the synaptic potentials produced by TTX was always accompanied by changes in the amplitude and latency of the l.o.t. compound action potential. Indeed, the depression of the population e.p.s.p. was proportional to the decrease in the amplitude of the l.o.t. compound action potential. Furthermore, the action of TTX on the action potential was accompanied by a dose-related increase in the electrical threshold of the l.o.t.

fibres and, in two preparations, there was also evidence of an increase in the firing threshold of the postsynaptic neurones. Neither of these two effects has ever been observed when low concentrations of pentobarbitone (0.1–0.3 mM) have been applied to the olfactory cortex, yet 0.3 mM pentobarbitone is more effective than 2×10^{-8} M TTX in depressing the evoked synaptic potentials (see Figure 2). The conclusion is therefore that pentobarbitone and TTX have different actions on synaptic transmission. Pentobarbitone appears to act principally on the mechanisms subserving chemical transmission (Weakly, 1969; Richards & Smaje, 1976) while TTX acts on the impulse generating mechanism in the efferent nerve axons. This conclusion is consistent with the observation that some general anaesthetics including pentobarbitone prolong and intensify inhibitory synaptic transmission (see, for example, Nicoll, Eccles, Oshima & Rubia, 1975).

The local anaesthetic, procaine, displayed some characteristics of the actions of both pentobarbitone and TTX. The most obvious initial change was a marked decrease in conduction velocity and increase in the electrical threshold of the l.o.t. fibres. For low concentrations of procaine (< 0.3 mM) the depression of the e.p.s.p. appeared to be directly related to the depression of the l.o.t. compound action potential but higher concentrations accentuated the depression of the e.p.s.p. These results suggest that procaine acts both on the impulse-generating mechanism of the afferent axons and on some part of parts of the mechanisms subserving chemical transmission. Consistent with this last point is the known depressant effect of procaine on the activity of the cholinoceptor of the neuromuscular junction (Ruff, 1977).

Since the time of Sherrington (1906) it has generally been believed that the actions of anaesthetics on synaptic transmission are the basis of the anaesthetic state. If this is so, the fact that local and general anaesthetics act on excitatory synaptic transmission by different mechanisms implies that there can be no single mechanism of anaesthetic action.

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