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An analysis of the action of pentobarbitone on the excitatory postsynaptic potentials and membrane properties of neurones in the guinea-pig olfactory cortex

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1 Intracellular recordings were made from neurones in slices of guinea-pig olfactory cortex maintained *in vitro* at 37°C. The average membrane potential was 63 ± 12 mV and the input resistance of these cells was 42 ± 20 MΩ (mean ± s.d.). Stimulation of the lateral olfactory tract (l.o.t.) generated a transient depolarization in these cells which had the characteristics of an excitatory postsynaptic potential (e.p.s.p.). If the e.p.s.p. was of sufficient amplitude it culminated in an action potential. The e.p.s.p. was potentiated by repetitive stimulation at 10–50 Hz and showed post-tetanic potentiation after a prolonged period of high frequency stimulation (50–100 Hz for 30–60 s).

2 Pentobarbitone (0.1–0.5 mM) depressed the e.p.s.p. reversibly but was without effect on the resting membrane potential, input resistance or time constant of the neurones. Pentobarbitone did not inhibit potentiation of the e.p.s.p. by a preceding conditioning shock.

3 It is concluded that pentobarbitone does not affect the passive membrane properties of neurones in the olfactory cortex. The depressant action of pentobarbitone on synaptic transmission results from a decrease in the amount of transmitter released in response to a nerve impulse, or a decrease in the sensitivity of the postsynaptic membrane to the transmitter or a combination of both effects.

Introduction

Direct actions of barbiturates on the resting membrane potential of CNS neurones have been reported (Nicoll, 1975; Nicoll & Madison, 1982; Scholfield, 1978c). These have led Scholfield (1980) to suggest that an increase in the resting membrane conductance may be the cause of the well-known depressant action of these drugs on excitatory synaptic transmission. While barbiturates have no effect on the resting membrane conductance of sympathetic neurones (Nicoll & Iwamoto, 1978) or spinal motoneurones (Weakly, 1969), their action on the resting membrane conductance of neurones in the mammalian brain is not known. The action of barbiturates on excitatory synaptic transmission in the brain has been studied by detailed analysis of field potentials recorded from the olfactory cortex (Richards, 1972b) but this technique may not be sufficiently sensitive to permit detection of modest changes in the passive membrane properties of neurones. To resolve this issue we have examined the action of pentobarbitone on the resting membrane potential, resting membrane conductance and excitatory postsynaptic potentials (e.p.s.ps) of neurones in slices of guinea-pig olfactory cortex maintained *in vitro*. A preliminary account of our results has already appeared (Richards & Strupinski, 1984).

Methods

Details of the methods of preparation, incubation and stimulation of slices of olfactory cortex have been given elsewhere (Richards, 1981). Briefly, guinea-pigs (250–400 g) were stunned by a blow to the back of the neck, and the spinal cord severed with a pair of large scissors. The brain was removed and slices of olfactory cortex were cut with a razor strip and a glass template. The slices had a nominal thickness of 350 μm and were incubated at 37°C in the chamber described by Richards & Tegg (1977). This chamber permits recordings to be made from slices immersed in a stream of oxygenated artificial cerebrospinal fluid (a.c.s.f.). Slices were placed in the recording chamber immediately after removal from the brain but recording

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started only after they had been left to recover from the trauma of isolation for at least 60 min.

The slices were stimulated by a pair of silver wires, insulated except at their tips, that were placed across the lateral olfactory tract (l.o.t.) close to its origin. Stimuli were of 50 μs duration and of varying intensity. They were delivered at intervals of 5–60 s. Intracellular records were obtained with high resistance glass micropipettes (65–250 MΩ; mean 150 ± 50 MΩ, $n = 38$) filled with 3M potassium acetate. The electrodes were coupled via a silver-silver chloride half-cell to a d.c. amplifier with a bridge circuit (WPI Model 707) to permit the passage of current into the impaled cell for monitoring input resistance. The membrane potential was monitored continuously with a digital voltmeter and the membrane resistance monitored by applying a hyperpolarizing pulse of 0.1–0.2 nA, amplitude and 40–100 ms duration shortly before or after the stimulus to the l.o.t. The data were stored on magnetic tape and subsequently analysed off-line with a digital oscilloscope and pen recorder.

All of the cells selected for detailed analysis of the action of pentobarbitone had stable membrane potentials in excess of 45 mV (usually > 60 mV) and action potentials that were greater than 50 mV in amplitude and less than 3 ms in duration.

The a.c.s.f. used to bathe the preparations had the following composition (in 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 and had a pH of 7.4 at 37°C. The pentobarbitone was dissolved in the a.c.s.f. at 0.1–0.5 mM and did not significantly change its pH.

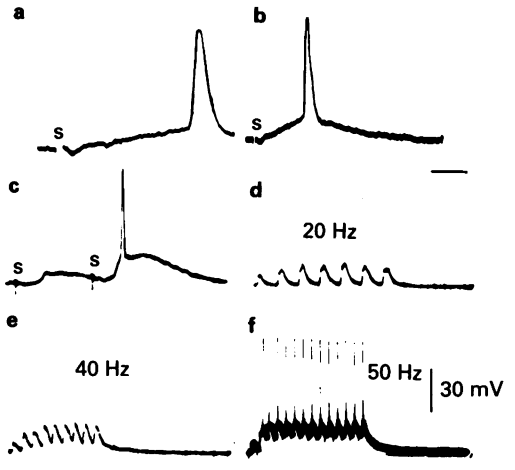

Figure 1 Examples of the intracellular records from two prepiriform neurones to show the synaptic responses to lateral olfactory tract (l.o.t.) stimulation. (a) and (b) The response of a prepiriform neurone to l.o.t. stimulation. Note the brief delay before the onset of the e.p.s.p., the action potential and the absence of any slow after-potential. (c) and (d) Examples of the potentiation and summation of e.p.s.ps in another prepiriform neurone. (c) The potentiation of the e.p.s.p. by a conditioning volley. Note the single action potential on the second (test) e.p.s.p. (d) – (f) The effect of repetitive stimulation on the amplitude of the e.p.s.p. and the sustained depolarization during high-frequency stimulation (temporal summation; e and f). (f) Shows cell discharge during the summation of the e.p.s.p. Horizontal calibration bar: 2 ms (a); 4 ms (b); 10 ms (c). Spikes retouched in (a), (b) and (c).

Results

Physiological properties of olfactory neurones

The resting membrane potential and input resistance of those neurones that met our criteria for satisfactory impalement were 63 ± 12 mV and 42 ± 20 MΩ (mean ± s.d.; $n = 34$). There was no obvious correlation between the value for the input resistance and the membrane potential in these cells ($r = 0.091$, $P > 0.7$). The membrane time constant was determined in 20 cells and averaged 15.8 ms (the range was 7.6–31.6 ms).

Stimulation of the l.o.t. with submaximal shocks generated a transient depolarization of 1–11 mV and 15–40 ms duration (the mean value was 30 ms). The depolarization began 1–2 ms after the stimulus and reached its peak value 5–7 ms after onset. If the strength of the l.o.t. volley was increased, the depolarization culminated in an action potential (see Figure 1 a – c). These characteristics are those expected of a monosynaptic e.p.s.p. Of the 30 cells in which the e.p.s.p. was sufficiently intense to generate an action potential 6 showed an after-hyperpolarization of 2–4 mV amplitude and 60–110 ms duration. The e.p.s.p. was potentiated on repetitive stimulation (Figure 1d) and showed temporal summation when stimulated at frequencies greater than 40 Hz (see Figure 1e, f). Prolonged stimulation (> 30 s) at 50 or 100 Hz resulted in post-tetanic potentiation of the e.p.s.p. that lasted about 1 min (not shown). The e.p.s.p. was also potentiated if it was preceded by another with a conditioning interval of 10–200 ms (Figure 1c). These characteristics of the intracellularly recorded e.p.s.p. agree closely with the changes in the evoked field potentials recorded under similar conditions (see Richards, 1972a).

Action of pentobarbitone on the e.p.s.p. and membrane potential

To examine the influence of pentobarbitone on the e.p.s.p. without interference from either i.p.s.p.s or

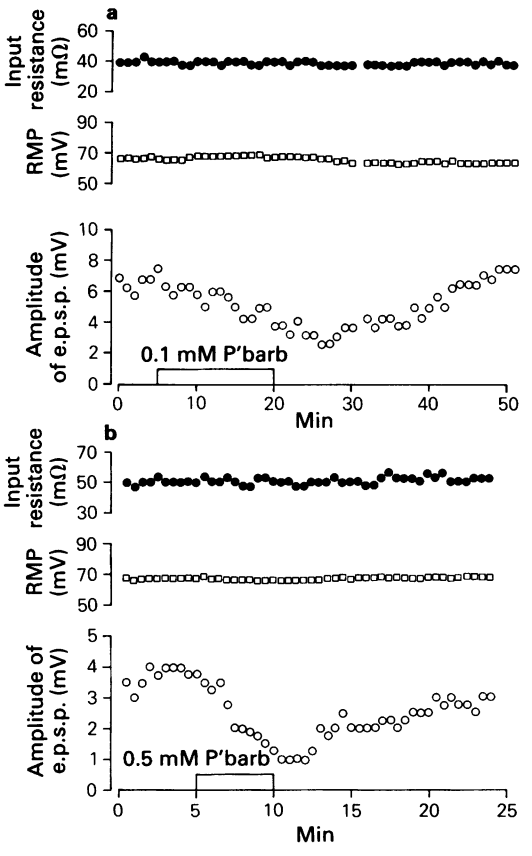

Figure 2 The time course of the action of pentobarbitone on the evoked e.p.s.p. recorded from two neurones in the prepiriform cortex. (a) Shows the effect of 0.1 mM pentobarbitone and (b) the effect of 0.5 mM. Note that the onset of pentobarbitone action in (b) was more rapid than in (a). The resting membrane potential (RMP) and input resistance were monitored throughout both experiments and showed no changes related to the application of the anaesthetic.

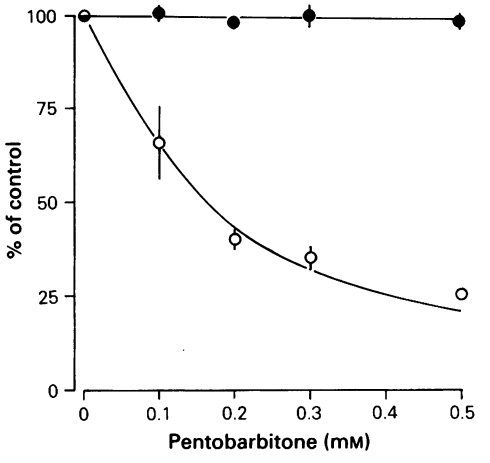

Figure 3 Differential action of pentobarbitone on the e.p.s.p. (○) evoked by lateral olfactory tract (l.o.t) stimulation and the resting membrane potential (●). The dose-response relationship for the action of pentobarbitone on the e.p.s.p. is approximate. In particular, the points for 0.5 mM pentobarbitone underestimate the final depression as this dose was applied for short periods only to avoid protracted recovery times (see Figure 2). Each point is the mean of 3–6 observations. The error bars indicate the standard errors. Where no bars are given the standard errors are similar in size to the symbols.

action potentials, the intensity of the l.o.t. volley was adjusted so that the e.p.s.p. did not evoke an action potential. So, under the conditions of our experiments, the l.o.t. volleys were generally submaximal. When pentobarbitone (0.1–0.5 mM) was added to the superfusion medium the e.p.s.p. became depressed. This effect was rapid in onset (within 2–5 min) and reached a maximum after 5–10 min. The effect was reversed on washing out the drug although full recovery took 10–15 min. Examples of the time course of the action of pentobarbitone can be seen in Figure 2.

In 20 trials on 12 cells the membrane potential was 99 ± 4% of its control value during the application of the anaesthetic. Although the effect of pentobarbitone was related to the concentration applied, there was no concomitant effect on the resting membrane potential (see Figure 2). In this respect the anaesthetic selectively affected the e.p.s.p. at all concentrations tested (see Figure 3).

The action of pentobarbitone on the potentiation of the e.p.s.p. was examined in 5 cells. Pairs of pulses 30 or 40 ms apart were used to stimulate the l.o.t. Pentobarbitone did not block the potentiation of the second (test) e.p.s.p. and did not appear to exert a preferential action on either the test or conditioning response (Figure 4).

Action of pentobarbitone on input resistance and time constant

The action of pentobarbitone on membrane resistance was assessed by monitoring the input resistance and time constant of olfactory neurones before, during and after exposure to the drug. The input resistance and time constant were monitored by measuring the amplitude and rate of rise of the change in membrane potential generated by a hyperpolarizing pulse of fixed

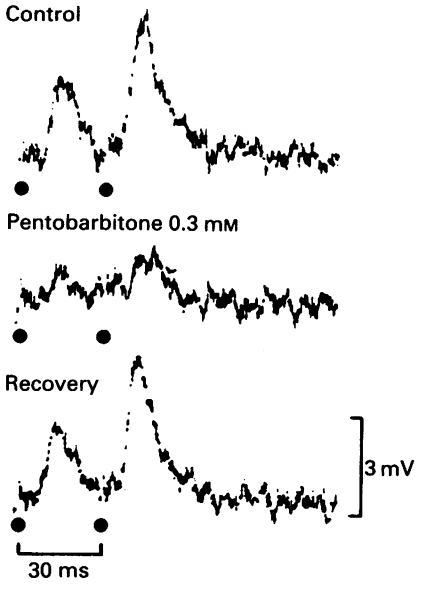

Figure 4 An example of the effect of pentobarbitone on the evoked e.p.s.p. recorded from a neurone in the prepiriform cortex. Two pulses, 30 ms apart (as indicated by the filled circles), were delivered to the lateral olfactory tract (l.o.t.) every 12 s. The second (test) e.p.s.p. was strongly potentiated. Pentobarbitone (0.3 mM) appeared to depress both test and conditioning e.p.s.ps to a similar extent.

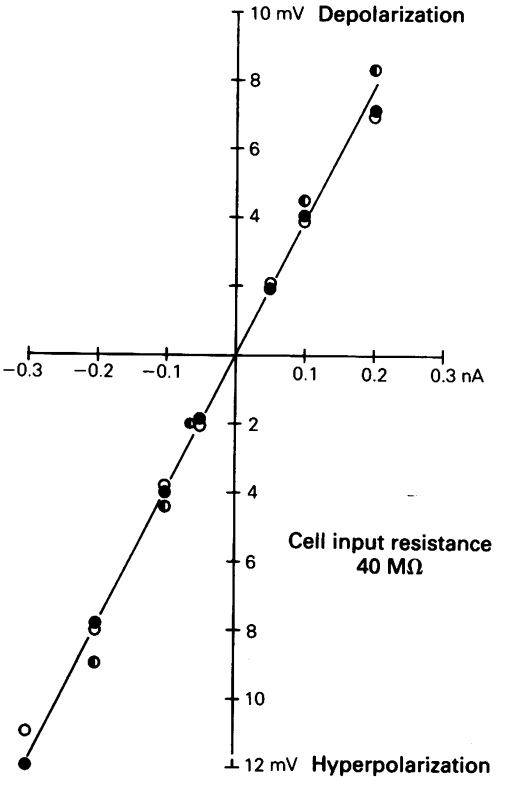

Figure 5 The action of pentobarbitone on the current-voltage relation for a neurone in the prepiriform cortex. The potential change is plotted as a function of the applied current. The input resistance of this cell was 40 MΩ and its resting membrane potential was −70 mV. (○) Control; (●) 0.2 mM pentobarbitone; (◐) recovery after washing out the drug.

amplitude and duration (0.1–0.2 nA, 40–100 ms). Pentobarbitone (0.1–0.5 mM) did not alter the amplitude of the hyperpolarizing pulse (see input resistance measurements in Figure 2). This was confirmed in 11 cells. Detailed analysis of the action of the drug on the time constant of 3 cells also failed to reveal any significant change.

Current-voltage curves were constructed for 7 prepiriform neurones. They were linear in the hyperpolarizing direction for 10–15 mV. In the depolarizing direction they were also linear for 8–10 mV but became non-linear with greater depolarizations. For the purposes of our study we confined our recordings to the linear portion of the curve. In no case did pentobarbitone (0.1–0.2 mM) alter the input resistance calculated from the slope of the current-voltage relationship (see Figure 5) even though it depressed the e.p.s.p. in all cells examined. As 0.3–0.5 mM pentobarbitone had no obvious effect on the input resistance of neurones in the olfactory cortex (see Figure 2b), the action of higher concentrations of pentobarbitone on current voltage curves were not explored.

Discussion

The synaptic properties of prepiriform neurones reported here correspond with those recorded *in vivo* in the cat (Biedenbach & Stevens, 1969) and the opossum (Haberly, 1973). The membrane potential recorded from olfactory neurones *in vitro* was higher than that recorded *in vivo*. This presumably reflects better impalement due to the greater stability afforded by the *in vitro* technique. The other basic electrophysiological characteristics of these cells (input resistance, time constant, duration and amplitude of the action potential) reported here are similar to those reported for olfactory neurones (Scholfield, 1978a) and for hippocampal neurones (Brown *et al.*, 1981).

Under the conditions of our experiments none of the

cells showed prolonged depolarizing after-potentials of the kind reported by Scholfield (1978b) and only 6 of a total of 30 cells showed hyperpolarizing i.p.s.ps. The absence of inhibitory synaptic potentials in most cells is probably the result of the severing of intracortical connections during preparation of the slices. This, together with the low frequency of stimulation, and the absence of any spontaneous activity has enabled us to analyse the action of pentobarbitone on excitatory synaptic transmission without interference from active inhibitory mechanisms.

As pentobarbitone depresses the e.p.s.p. field potentials without significantly affecting conduction in the afferent nerve fibres (see Richards, 1972b; 1982), it must be acting either on the process of chemical transmission or on the resting membrane to limit the spread of synaptic current. Here we have shown that pentobarbitone depresses the e.p.s.p. without any measurable effect on the passive membrane properties or the resting membrane potential of the postsynaptic cells. The depression of excitatory synaptic transmission is, therefore, not the result of an increase in resting membrane conductance as Scholfield (1980) has suggested.

Direct effects of pentobarbitone on the membrane potential have been reported by some workers (Nicoll, 1975; Scholfield, 1978c; Nicoll & Madison, 1982). These have been ascribed to its action on receptors for inhibitory transmitters such as γ-amino butyric acid. We found no evidence of this in our study although we used concentrations of pentobarbitone that span the anaesthetic range (see Richards, 1972b). The reason for this discrepancy is not clear but the results indicate that such direct actions are not a general feature of anaesthetic action on central neurones (see also Sawada & Yamamoto, 1985).

To conclude, pentobarbitone appears to depress excitatory synaptic transmission in the CNS by a direct action on the process of chemical transmission and not by an increase in the resting membrane conductance. It does not appear to modulate the resting membrane potential. Exactly how it acts on the synapse remains an open question but both presynaptic (Weakly, 1969, Collins, 1980) and postsynaptic (Richards & Smaje, 1976; Sawada & Yamamoto, 1985) mechanisms have been implicated.

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