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PENTOBARBITONE INTERFERENCE WITH INHIBITORY SYNAPTIC TRANSMISSION IN CRAYFISH STRETCH RECEPTOR NEURONES

BY C. CLAIRE AICKIN* AND R. A. DEISZ†

From the Department of Neurophysiology, Max-Planck-Institute for Psychiatry, D-8000 Munich 40, Federal Republic of Germany

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SUMMARY

1. The effect of pentobarbitone (PB) on GABA-ergic inhibition was investigated in the isolated crayfish stretch receptor. The soma of the slowly adapting neurone was impaled with two micro-electrodes to give an accurate determination of membrane conductances.

2. Application of PB in concentrations from 10^{-6} to 10^{-3} M increased the rise time constant of the inhibitory post-synaptic potential (i.p.s.p.). The i.p.s.p. percentage amplitude and decay time constant were also increased in eight out of twelve neurones. On prolonged exposure, the percentage amplitude declined at a rate dependent upon the dose and the frequency of stimulation until the i.p.s.p. became undetectable.

3. The response to ionophoretically applied GABA remained essentially unaltered in the presence of PB, but the falling phase was prolonged by up to 8% in four of the ten neurones tested. Resting membrane conductance, i.p.s.p. driving force (i.p.s.p. reversal potential minus resting membrane potential), and parameters of the anti- and orthodromic action potential were not significantly affected.

4. Removal of PB after prolonged exposure usually caused an immediate increase in i.p.s.p. percentage amplitude but the i.p.s.p. rising phase remained slowed.

5. Application of excess extracellular GABA only affected the i.p.s.p. percentage amplitude after it had been reduced by PB. It transiently increased the attenuated i.p.s.p. percentage amplitude in the presence of PB, and after the removal of PB permanently increased the amplitude to its original value.

6. Nipecotic acid and *cis*-1,3-aminocyclohexane carboxylic acid, inhibitors of GABA re-uptake, slightly increased the i.p.s.p. percentage amplitude, and prolonged the falling phase but did not affect the rising phase. The percentage amplitude declined on prolonged exposure.

7. We conclude that PB has no electrophysiologically demonstrable post-synaptic action in the crayfish stretch receptor neurone, but it inhibits the presynaptic release and re-uptake of GABA.

* Present address: University Department of Pharmacology, South Parks Road, Oxford OX1 3QT.

† To whom reprint requests should be addressed.

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INTRODUCTION

In 1970, Larsen & Major observed prolongation of the inhibitory post-synaptic potential (i.p.s.p.) by barbiturates in cat spinal motoneurones and thus resolved the discrepancy between the recurrent inhibition time measured from reflexes in the decerebrate animal and that measured by intracellular recording in the anaesthetized animal. Since then there have been many corroborative reports, including description of a fivefold (Nicoll, Eccles, Oshima & Rubia, 1975) or even a tenfold (Scholfield, 1978) increase in the i.p.s.p. duration. Other effects have been described, but the dominant action appears to be alteration of synaptic transmission, with depression of excitatory synaptic potentials also reported in both mammalian (Richards, 1972; Nicoll, 1972) and non-mammalian preparations (Barker & Gainer, 1973; Proctor & Weakly, 1976). However, the underlying mechanism of barbiturate interference with synaptic transmission, and particularly i.p.s.p. prolongation by pentobarbitone (PB), is still in contention despite the wealth of literature, obtained mainly in mammalian preparations. A post-synaptic mechanism is perhaps the most favoured (e.g. Jessel & Richards, 1977; Barker & Ransom, 1978; Brown & Constanti, 1978; Lodge & Curtis, 1978, Scholfield, 1978; Ticku & Olsen, 1978), and some authors have even reported a GABA-mimetic action of PB (Nicoll, 1975; Nicoll & Iwamoto, 1978; MacDonald & Barker, 1978; Evans, 1979).

The major evidence for a post-synaptic action has been provided indirectly, by experiments in which GABA was applied artificially. Potentiation of the response under these conditions is most simply explained by either a post-synaptic action or delayed removal of GABA by inhibition of the uptake mechanism. Since nipecotic acid, an inhibitor of GABA uptake (Johnston, Krogsgaard-Larsen & Stephanson, 1975), failed to duplicate all the observed PB effects (Bowery & Dray, 1977; Brown & Constanti, 1978; Evans, 1979), potentiation was considered unlikely to have been mediated by the latter mechanism. Further evidence for a post-synaptic action has been interpreted from the reversal of the action of GABA antagonists by PB, first reported for picrotoxin (PTX) by Eccles, Schmidt & Willis (1963) and later for bicuculline by Bowery & Dray (1976). Subsequently there have been many electrophysiological reports confirming this PB–GABA antagonist interaction (e.g. Curtis & Lodge, 1977; Barker & Ransom, 1978; Scholfield, 1978; Evans, 1979). PB has also been shown to inhibit the binding of α-dihydropicrotoxinin, a biologically active analogue of PTX (Ticku, Ban & Olsen, 1978).

The reported, pronounced i.p.s.p. prolongation, reversal of PTX antagonism, and the suggested PB mechanism (recently confirmed for phenobarbitone: Barker & McBurney, 1979) of increasing the mean open time of the GABA-activated channels in mammalian preparations (Lodge & Curtis, 1978; Ticku & Olsen, 1978) are remarkably like the effects and proposed mechanism of 5,5-diphenylhydantoin (DPH) in the crayfish stretch receptor (Deisz & Lux, 1977*a*, *b*; Adams, Banks & Constanti, 1981; Aickin, Deisz & Lux, 1981). Thus it seemed likely that PB might act at the same site as DPH, a possibility supported by their similarity in chemical structure. Therefore we have determined the effect of PB on both the evoked i.p.s.p. and the response to ionophoretically applied GABA in the slowly adapting neurone of the isolated crayfish stretch receptor. The anticipated PB–PTX antagonism was

also investigated. The results reveal a completely unexpected effect which may help to elucidate the action of PB.

A preliminary report of some of these results has been communicated to the Physiological Society (Aickin & Deisz, 1979).

METHODS

These methods were the same as described in the foregoing paper (Aickin *et al.* 1981) with the exception that the experiments were performed at 17·5 °C. The higher temperature was used because it seemed to facilitate the i.p.s.p. recovery following PB removal. Only about one in twenty neurones was acceptable in this series of experiments since an optimal response to ionophoretically applied GABA was required in addition to the criteria given previously (Aickin *et al.* 1981). PB (Nembutal, Abbott S.A.), nipecotic acid and *cis*-1,3-aminocyclohexane carboxylic acid (gift from Dr N. G. Bowery) were added directly to the normal Ringer solution to give the required concentrations.

RESULTS

Effect of PB on the evoked i.p.s.p.

In contrast to the consistent increase in i.p.s.p. amplitude and decay time constant on application of a suprathreshold concentration of DPH (Deisz & Lux, 1977*a*, *b*; Aickin *et al.* 1981), PB had these effects in only eight out of twelve neurones. But it always prolonged the rising phase, and unexpectedly caused a progressive decline in amplitude on continued exposure to concentrations between 10^{-6} and 10^{-3} M. The sequence of effects is shown in Fig. 1, in a neurone where PB initially increased the i.p.s.p. percentage amplitude (i.p.s.p. amplitude expressed as a percentage of the difference between the membrane potential at which it was elicited and the i.p.s.p. reversal potential: see Deisz, Aickin & Lux, 1979). Both the rise and decay time constants were increased beyond the prevailing membrane time constants (see Aickin *et al.* 1981). Following the initial increase the i.p.s.p. percentage amplitude steadily declined and after 25 min was virtually the same as before PB application. Comparison of the i.p.s.p.s recorded at this time with those in the control period well illustrates the slowed rising and falling phases. The i.p.s.p. continued to decline until, 145 min after PB application, it was no longer detectable.

The rate of decline was dose-dependent for each neurone, but showed great variation for the same dose in different neurones at the same frequency of stimulation. For example, the time course of the decline in the neurone illustrated in Fig. 1 during exposure to PB 10^{-3} M was slower than that observed in some other neurones during exposure to 10^{-6} M. This difference was not correlated with the initial i.p.s.p. percentage amplitude. The rate of decline was also dependent upon the frequency of stimulation, a slower decline being observed with a lower frequency. Removal of PB following a long exposure usually caused an immediate increase in percentage amplitude, but the PB-induced slowing of the rising phase was irreversible, at least within 2 hr of washout. Resting membrane potential (E_m) and i.p.s.p. reversal potential ($E_{i.p.s.p.}$) were not systematically affected. The i.p.s.p. driving force ($E_{i.p.s.p.}$ minus E_m) and the time to peak, overshoot and total duration of the anti- and orthodromic action potential remained virtually unaltered throughout prolonged exposure to PB at concentrations between 10^{-6} and 10^{-3} M, and after its removal.

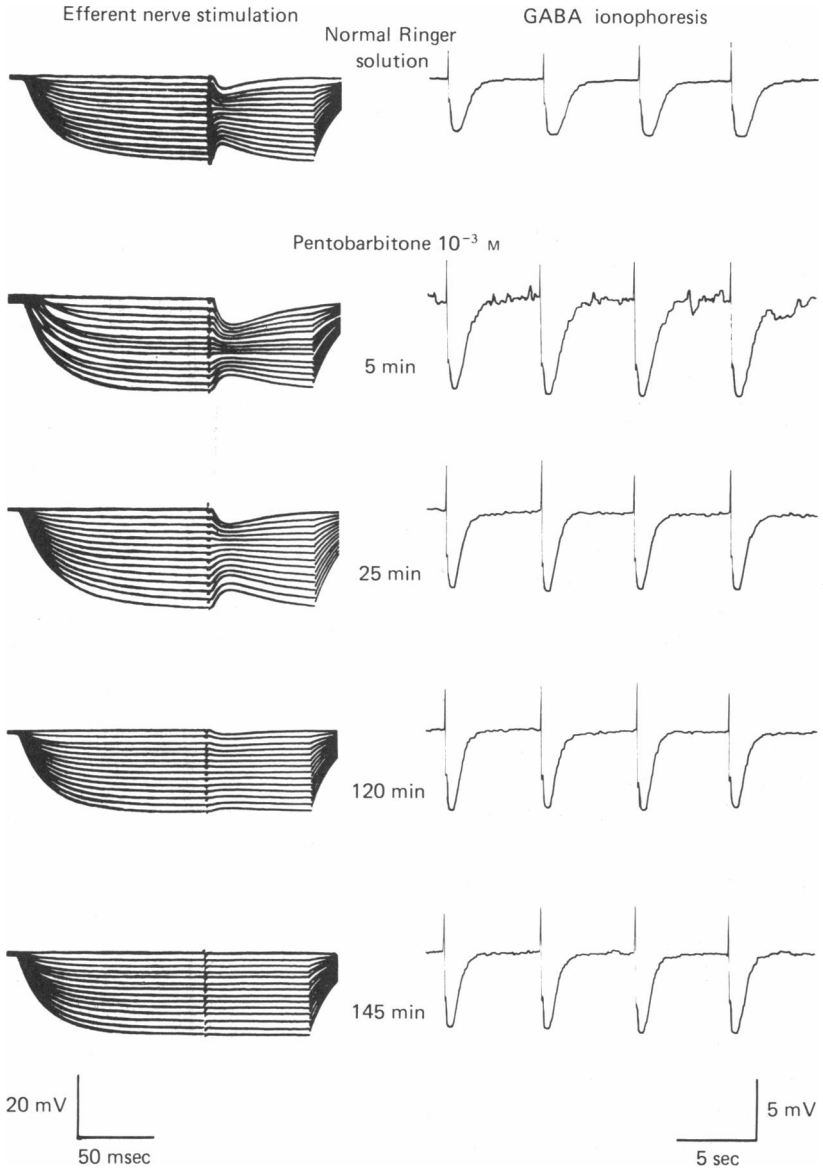

Fig. 1. Oscillographs of the i.p.s.p. (time of stimulation obvious from the stimulus artifact) evoked at various membrane potentials, obtained by injection of increasing current steps (each of 0·5 nA), and pen recordings of the response to ionophoretically applied GABA (time of application obvious from the current artifact; pulse duration 20 msec). Both were obtained in normal Ringer solution immediately before PB application and at the times indicated after its application. The times apply strictly to the oscillographs, the pen recordings being taken about 1 min later. Mean resting membrane potential was 58·5 mV in normal Ringer solution and 49, 58, 55 and 53 mV at 5, 25, 120 and 145 min after PB application respectively. The neurone depolarized with every solution change in this experiment, probably as a result of induced movement of the preparation. It fired spontaneously for 6 min on application of PB and then repolarized slowly. This accounts for the irregular rate of attainment of the steady-state membrane potential on current

Resting membrane conductance was not significantly affected but showed the continuous decline with time characteristic of these neurones (Deisz *et al.* 1979) both during PB application and after its removal.

Effect of PB on the response to ionophoretically applied GABA

These effects on the evoked i.p.s.p. could be due to pre- or post-synaptic mechanisms, or to a combination of both. Thus we investigated the effect of PB on the post-synaptic response by applying GABA ionophoretically. Although comparison of ionophoretic response over a long period is notoriously difficult, apparently reliable results can be obtained in this preparation, as judged from the comparable change in the ionophoresis response and evoked i.p.s.p. on application of DPH or PTX (Aickin *et al.* 1981). The kinetics of the resultant voltage change were essentially unaltered by the presence of PB, as shown in Fig. 1, both when the i.p.s.p. was potentiated and when it was no longer detectable. The time constant of the falling phase was marginally increased, by up to 8 %, in four out of ten neurones. The increase in the amplitude of the response throughout the PB exposure shown in Fig. 1 was not consistently observed and in fact in six of the ten neurones there was a slight decrease. This change in amplitude was probably due to alteration in the relative position of the ionophoresis electrode during the solution change (see legend to Fig. 1).

The virtually unaltered response to ionophoretically applied GABA in the presence of PB suggests little if any post-synaptic action. Further, the observation that DPH 10^{-4} or 10^{-5} M caused a considerable prolongation and increase in amplitude of both the i.p.s.p. and GABA ionophoresis response when the i.p.s.p. amplitude had dwindled to about 10 % in the presence of PB suggests that PB does not share the post-synaptic mechanism of DPH.

Effects of excess extracellular GABA on the PB-induced decline in i.p.s.p. amplitude

The above results make a post-synaptic action of PB unlikely, thus implying that the effects on the evoked i.p.s.p. were presynaptically mediated. The substantially slowed i.p.s.p. rising phase, together with the immediate partial recovery in i.p.s.p. amplitude on removal of PB, indicates that GABA release might have been affected. However, altered release would only account for the progressive decline in i.p.s.p. percentage amplitude if there was a slowly developing inhibition. The rundown is more readily explained by inhibition of GABA re-uptake. This would cause a gradual depletion of the presynaptic content provided that *de novo* synthesis did not sufficiently compensate for the loss.

Fig. 2 shows an experiment designed to test this possibility. The i.p.s.p. percentage amplitude increased on application of PB and then, as usual, progressively declined. Alteration of the determination interval from 1 to 2 min caused a reversible decrease in the rate of decline. A series of 100 GABA ionophoresis pulses were applied when

injection in the oscillograph taken at 5 min and for the fluctuations in the following pen recordings. Disturbance of the preparation during the solution change may also have caused a slight change in the location of the tip of the ionophoresis electrode and thus in the amplitude of the response. The current electrode fell out following PB removal and subsequent attempts at re-penetration killed the neurone. However, before the attempts were made, the i.p.s.p. was comparable to that observed between 25 and 120 min after PB application and the ionophoresis response was unaltered.

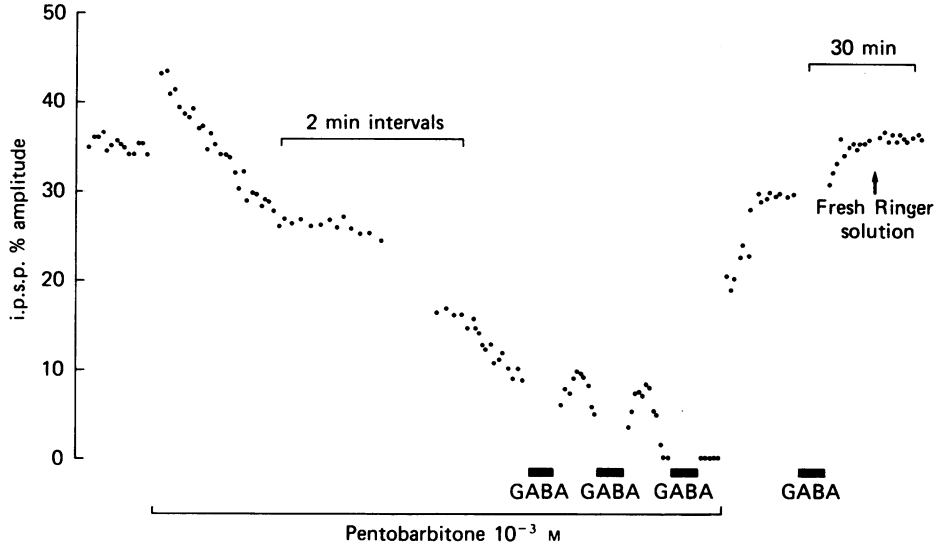

Fig. 2. Plot of the i.p.s.p. percentage amplitude *versus* time showing the effect of alteration of the determination interval from 1 to 2 min in the presence of PB 10^{-3} M and of application (7 min duration) of excess extracellular GABA during and after exposure to PB. The percentage amplitude was determined from i.p.s.p.s elicited during fourteen current steps. Determinations were made at 1 min intervals except for the period indicated, during positioning of the ionophoresis electrode (break in the plot after about 1 hr in PB), repetitive ionophoresis and solution changes. Since solution changes caused considerable movement of the preparation in this experiment, the usual procedure of exchanging the solution every 15 min was not adhered to, and changes were kept to a minimum. After the ionophoresis electrode had been successfully positioned, i.p.s.p.s could not be elicited with the previous stimulation parameters; the suction and intensity of stimulation had to be increased. This may account for the fall in percentage amplitude of about 8%. Alternatively, positioning of the ionophoresis electrode may have distorted the dendrites and branches of the efferent nerve. GABA was applied by 100 ionophoresis pulses at the intervals indicated by the bars. Fresh normal Ringer solution was applied to remove any residual extracellular GABA at the time indicated. PB 10^{-3} M was used in this experiment since an earlier application at 10^{-5} M had shown that the i.p.s.p. was particularly resistant to depression by PB. The same effects have been observed in other, more sensitive neurones at 10^{-6} M and it is likely that they would have been observed in this neurone at lower concentrations,but over a much longer period.

the i.p.s.p. amplitude had declined to about 15%. Immediately after the period of ionophoresis the percentage amplitude was higher than if the decline had continued unaltered. It further increased for another 5 min, but then declined again at a faster rate than before. A second series of GABA pulses was then applied and the percentage amplitude again increased in the following 6 min. Thereafter, it once more declined and became unmeasurable. A further 100 GABA ionophoresis pulses failed to restore the i.p.s.p. to a detectable level. PB was then removed from the bath and the i.p.s.p. immediately re-appeared, at about 20%. The percentage amplitude improved over the next 6 min and stabilized at about 30%, 5% lower than initially recorded before PB exposure. A series of 100 GABA pulses was again applied and, as in the presence of PB, the percentage amplitude immediately increased and continued to increase for 5 min. But unlike in the presence of PB, it then remained stable, in this case at

the control level (35%). This improvement in the i.p.s.p. amplitude was not due to residual extracellular GABA since the percentage amplitude remained unaltered, or even slightly increased, after application of ample fresh Ringer solution (sixty bath volumes). Further repetitive ionophoresis (not shown in Fig. 2) did not augment the percentage amplitude above the control level.

To ensure that excess extracellular GABA did not increase the percentage amplitude under normal conditions, we have both applied GABA by repetitive ionophoresis and applied it directly to the bath at 5×10^{-4} M before application of PB. The i.p.s.p. percentage amplitude was not significantly affected. In some experiments, repeated GABA ionophoresis failed to increase the i.p.s.p. percentage amplitude after it had declined in PB. But in these cases the i.p.s.p. could be improved by a 5 min bath application of GABA 5×10^{-4} M. However, when the i.p.s.p. had become undetectable it could not be restored by either repetitive ionophoresis, when previously effective, or bath application of GABA (ten neurones).

The occasional failure of repetitive ionophoresis to augment the attenuated, but still detectable i.p.s.p., both in the presence of PB and after its removal, is probably explained by the very localized application of GABA; the post-synaptic response was usually lost by movement of the tip of the ionophoresis electrode by approximately 5 μm. It is possible that the presynaptic terminals in the region of the ionophoresis electrode were not activated by stimulation of the efferent nerve due to branch block.

Effect of GABA re-uptake inhibitors on the evoked i.p.s.p.

Because these results suggest that PB inhibits GABA re-uptake, known blockers of the re-uptake system were tested. The drugs used were nipecotic acid, reported to affect both neuronal and glial re-uptake systems (Bowery, Jones & Neal, 1976), and *cis*-1,3-aminocyclohexane carboxylic acid, a specific blocker of neuronal uptake (Bowery *et al.* 1976). They both induced a slight increase in i.p.s.p. percentage amplitude, as shown in Fig. 3, and a marginal prolongation of the i.p.s.p. falling phase. The i.p.s.p. rising phase was unaffected. Continued exposure to either drug caused a progressive decline in the percentage amplitude. Following drug removal, the percentage amplitude immediately increased and stabilized at a lower level than recorded before application. In the experiment shown in Fig. 3 application of PB at the same concentration had the same effects with the exception of increasing the i.p.s.p. rise time constant. The increase in percentage amplitude and subsequent rate of decline, on application of the same concentration of each drug at the same frequency of stimulation, was greatest with *cis*-1,3-aminocyclohexane carboxylic acid and approximately equal with nipecotic acid and PB (see Fig. 3).

Effect of PB on the evoked i.p.s.p. in the presence of PTX

Although the PB effects described so far show few of the characteristics reported in mammalian preparations, it seemed possible that investigation of PB reversal of PTX–GABA antagonism (Eccles *et al.* 1963) might reveal some. In addition, comparison with the interaction between DPH and PTX reported in the foregoing paper (Aickin *et al.* 1981) would indicate whether there are any common features in the actions of PB and DPH. These were expected from the equivalent mechanisms proposed for PB in mammalian preparations (Lodge & Curtis, 1978; Ticku & Olsen, 1978) and DPH in this preparation (Deisz & Lux, 1977*a*; Adams *et al.* 1981; Aickin

et al. 1981) and from the report of PB- and DPH-induced inhibition of α-dihydropicrotoxinin binding at both the rat brain (Ticku *et al.* 1978) and crayfish muscle (M. K. Ticku, personal communication) GABA receptor–ionophore complex.

On application of PTX 10^{-5} M, the i.p.s.p. percentage amplitude decreases and the rising phase is prolonged (Aickin *et al.* 1981). Addition of PB 10^{-4} M in the presence of PTX 10^{-5} M slightly increased the i.p.s.p. percentage amplitude in two of the four neurones tested (from 16 to 20 % and from 12 to 17 %) and prolonged the falling phase, as shown in Fig. 4. But the i.p.s.p. rise time constant was further increased. PB 10^{-3} M

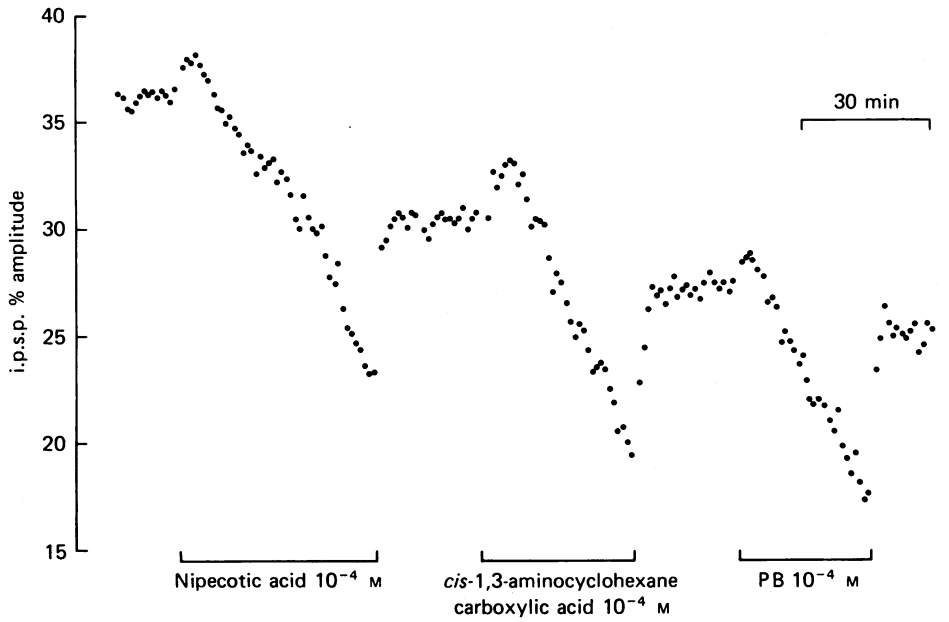

Fig. 3. Plot of the i.p.s.p. percentage amplitude *versus* time showing the effect of application of nipecotic acid 10^{-4} M, *cis*-1,3-aminocyclohexane carboxylic acid 10^{-4} M and PB 10^{-4} M. The percentage amplitude was determined from i.p.s.p.s elicited during fourteen current steps at 1 min intervals except for the periods of solution change.

was then applied in one of these neurones. No further increase in the i.p.s.p. amplitude was observed and after 10 min it began to decline. In the other two neurones the i.p.s.p. immediately decreased to an undetectable level. Fig. 4 also shows the effect of DPH 10^{-4} M application in the presence of PTX 10^{-5} M. As described in the preceding paper (Aickin *et al.* 1981) the i.p.s.p. percentage amplitude was considerably increased, in this case from 22 to 63 %, and the falling phase was substantially prolonged. However, in complete contrast to the additive effect of PB and PTX in increasing the i.p.s.p. rise time constant, DPH removed the PTX-induced increase, the rise time constant returning to the effective membrane time constant (see Aickin *et al.* 1981). These results suggest that PB has a completely different action from DPH.

DISCUSSION

The unprecedented, progressive decline in the i.p.s.p. on exposure to PB emphasizes the need for caution in proposing the mode of action of a drug from its short-term effects. Brief application of PB indicates a weak potentiation of the i.p.s.p. in many neurones, similar to the more pronounced effect seen in mammalian preparations (e.g. Nicoll *et al.* 1975; Scholfield, 1978). This, together with the marginal prolongation of the response to GABA ionophoresis sometimes observed, could be interpreted as evidence for a post-synaptic mechanism equivalent to that described for DPH (Deisz

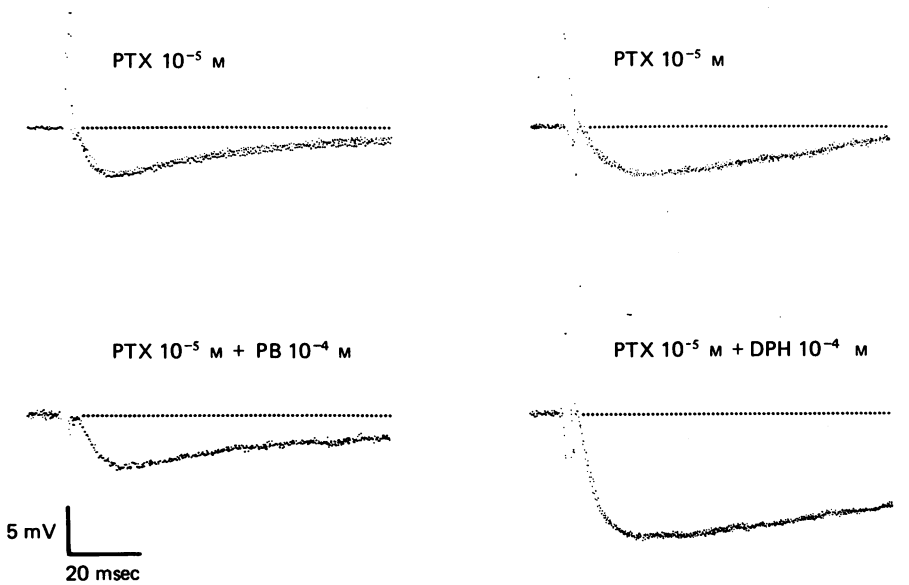

Fig. 4. Comparison of the effect of PB 10^{-4} M and DPH 10^{-4} M on the evoked i.p.s.p. in the presence of PTX 10^{-5} M. The recordings on the left were obtained in a neurone at a resting potential of 57 mV and resting membrane conductance of $2{\cdot}70 \times 10^{-7}$ S, and those on the right in another neurone at a resting potential of 62 mV and resting membrane conductance of $1{\cdot}32 \times 10^{-7}$ S. Each recording is composed of four superimposed i.p.s.p.s, displayed on an *xy* point plotter. The dotted lines represent the mean membrane potential.

& Lux, 1977*a*; Adams *et al.* 1981; Aickin *et al.* 1981). But the progressive decline in the i.p.s.p. percentage amplitude on prolonged PB exposure precludes such a simple hypothesis. It seems unlikely that a local anaesthetic action on the efferent nerve could explain the continuity of the decline. This is borne out to some extent by the unaltered anti- and orthodromic action potential recorded throughout prolonged PB exposure.

The decline in the i.p.s.p. could be due to a gradual depletion of the presynaptic GABA content through inhibition of the re-uptake mechanism. This is supported by the frequency dependence of the rate of decline. Inhibition of re-uptake would also probably delay the removal of GABA from the synaptic cleft and so could be responsible for the increase in i.p.s.p. percentage amplitude, and rise and decay time constants usually observed immediately on application of PB. The fact that i.p.s.p.

potentiation was not observed in every neurone was a little worrying but perhaps to be expected since slight prolongation of the synaptic current can be obscured by a relatively large membrane time constant (Aickin *et al.* 1981). The observation of the same changes in the i.p.s.p. amplitude and decay time constant on application of, and continued exposure to, nipecotic acid and *cis*-1,3-aminocyclohexane carboxylic acid, also supports the suggestion of PB-induced inhibition of GABA re-uptake. The increase in the attenuated i.p.s.p. percentage amplitude following application of excess extracellular GABA is not inconsistent with the suggestion of inhibited re-uptake, since the inhibition may not be complete. The residual GABA re-uptake would then partially restore the presynaptic content but only to an observable extent when GABA was present at higher concentrations for a longer period. The gradual improvement of the i.p.s.p. following application of an excess of extracellular GABA may be explained by the probable delay between the uptake of GABA and its subsequent availability for release. Although results from a variety of preparations indicate that PB only inhibits [^{3}H]GABA re-uptake in the millimolar range (Cutler, Markowitz & Dudzinski, 1974; Peck, Miller & Lester, 1976; Lodge & Curtis, 1978), electron microscopy and freeze etching have revealed marked changes in presynaptic structure after PB application at lower concentrations (Streit, Akert, Sandri, Livingston & Moor, 1972; Jones & Devon, 1978; Hajós, Csillag & Kálmán, 1978). These results have been interpreted as being due to blocked transmitter re-uptake (Jones & Devon, 1978).

But the effects of PB may not be entirely attributable to inhibition of GABA re-uptake. First, the immediate partial recovery of the i.p.s.p. on removal of PB indicates that depletion of the presynaptic GABA content was not solely responsible for the decline in percentage amplitude. This might suggest that GABA release was reversibly reduced by PB. However, removal of both nipecotic acid and *cis*-1,3-aminocyclohexane carboxylic acid after the i.p.s.p. percentage amplitude had declined also caused an immediate partial recovery. Although this too could be due to disinhibition of GABA release, these drugs have been suggested to increase GABA efflux (Bowery *et al.* 1976). An alternative explanation is on the basis of the presynaptic modulation of GABA release by negative feedback proposed by Mitchell & Martin (1978). When uptake is no longer inhibited, GABA is cleared from the synaptic cleft more rapidly again and so may exert a less effective feed-back on the release mechanism. Secondly, although the prolonged rising phase might be due to delayed removal of GABA from the synaptic cleft (see Katz & Miledi, 1973), this effect was not seen on application of nipecotic acid or *cis*-1,3-aminocyclohexane carboxylic acid. The suggestion of a PB-induced inhibition of release is in agreement with other reports (Cutler *et al.* 1974; Haycock, Levy & Cotman, 1977; Jessel & Richards, 1977; Coleman-Riese & Cutler, 1978) and could be due to inhibition of depolarization-induced calcium influx into the presynaptic terminals (Blaustein & Ector, 1975). In addition, this action could also account for the reported depression of excitatory transmission (e.g. Richards, 1972; Nicoll, 1972; Barker & Gainer, 1973). Although a less disputed mechanism, it equally cannot account for all the observed effects, particularly the improvement in the PB-declined i.p.s.p. following application of an excess of extracellular GABA.

Contrary to expectation, the only marginal prolongation of the response to

ionophoretically applied GABA demonstrates that PB has little, if any, post-synaptic action that might have been obscured by presynaptic effects. In fact the prolongation can be explained by inhibition of re-uptake. The conclusion of two presynaptic mechanisms seems difficult to equate with the majority of reports from mammalian preparations, where a post-synaptic mechanism is strongly favoured. The marginal prolongation of the i.p.s.p. on application of inhibitors of GABA uptake in the crayfish stretch receptor (see also Deisz & Lux, 1977*b*) compared with the considerable effects reported in mammalian preparations (Gottesfeld, Kelly & Renaud, 1972; Brown & Galvan, 1977; Brown & Constanti, 1978; Evans, 1979) suggests that the duration of the post-synaptic response in the latter is much more dependent on the rate of GABA removal, thus probably explaining the difference in the potency of PB-induced potentiation of the i.p.s.p. observed in mammals and invertebrates. It may be relevant that Bauer (1979) has reported a biphasic effect of PB on [^{3}H]GABA efflux from rabbit retina remarkably like the effects on the i.p.s.p. percentage amplitude reported here. These effects led her also to conclude that PB inhibits both GABA release and re-uptake. Finally, it is worth emphasizing that, at least in the crayfish stretch receptor, PB and DPH do not have equivalent mechanisms.

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