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Actions of Pentobarbital on Rat Brain Receptors Expressed in *Xenopus* Oocytes

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Functional receptor channels activated by GABA and other neurotransmitters were "transplanted" from rat brain to *Xenopus* oocytes by injecting the oocytes with total poly(A)$^+$ mRNA isolated from rat or chick brain. Membrane currents elicited in the oocyte by GABA inverted polarity at about the chloride equilibrium potential (ca. −25 mV). Pentobarbital potentiated the GABA-activated currents, without appreciably changing the reversal potential or form of the current-voltage relationship. At low (<10^{-5} M) concentrations of GABA, pentobarbital (100 μM) potentiated the responses by a factor of 10 or more, but responses to high (ca. 1 mM) concentrations of GABA were almost unchanged. Half-maximal activation of the response was obtained with about 3×10^{-5} M GABA when applied alone and with about 4×10^{-6} M GABA when applied together with 100 μM pentobarbital. At low doses of GABA, the size of the current increased as the 1.4th power of GABA concentration, but this relationship became nearly linear in the presence of pentobarbital. The potentiation of the GABA response increased linearly with concentrations of pentobarbital up to about 300 μM, reaching a maximum of about 50-fold. At higher concentrations of pentobarbital, the response to GABA declined. Relaxations of GABA-activated currents following voltage steps became slower in the presence of pentobarbital, suggesting that the open lifetime of the channels was prolonged. In addition to actions on GABA-activated currents, pentobarbital itself elicited a small membrane current that inverted polarity at a potential (−10 mV) more positive than the GABA-activated current. Also, responses elicited by activation of rat brain kainate receptors were depressed by pentobarbital, without changing the reversal potential or the form of the current-voltage relation.

Barbiturates are therapeutically important drugs, with sedative, antiepileptic, and anesthetic actions. Many studies have been made of their effects on neurons in the mammalian CNS, and it is well established that an important part of their action is the enhancement of synaptic inhibition mediated by GABA (Haefely et al., 1979; Willow and Johnston, 1983). Much remains to be discovered, however, and the electrophysiological study of barbiturate actions on central neurons is beset by many technical difficulties. The use of isolated or cultured neurons has provided one important technique allowing detailed investigation of barbiturate actions at a cellular level (Akaike et al., 1985; Barker and McBurney, 1979; Mathers and Barker, 1978; Ransom and Barker, 1975). A different approach to brain neurotransmitter receptors has recently become available, with the finding that many types of receptors can be "transplanted" to frog oocytes by injecting the oocytes with mRNA derived from brain (Gundersen et al., 1983a, 1984b; Houamed et al., 1984; Miledi et al., 1982; Sumikawa et al., 1984).

GABA receptors expressed in the oocyte by mRNA from chick and rat brain have properties similar to those in the brain (Miledi et al., 1982), and, in particular, the responses to GABA in the oocyte are potentiated by barbiturates and benzodiazepines (Gundersen et al., 1984c; Smart et al., 1983; Sumikawa et al., 1984). The investigation of brain receptors is greatly facilitated when they are expressed in this simplified cell system, and we describe here the effects of the anesthetic barbiturate pentobarbital on GABA and other neurotransmitter receptors transplanted to the oocyte from rat and chick brain.

Materials and Methods

Procedures for extraction of poly(A)$^+$ mRNA from brains of adult Wistar rats or chick embryo optic lobe and its injection into oocytes of *Xenopus laevis* were as described previously (Gundersen et al., 1983b, 1984a; Miledi and Sumikawa, 1982; Miledi et al., 1982). Oocytes were voltage-clamped using a 2-electrode system (Kusano et al., 1982; Miledi, 1982) and were continuously superfused with Ringer's solution (NaCl, 115 mM; KCl, 2 mM; $CaCl_2$, 1.8 mM; HEPES, 5 mM, at pH ~7.2) via a gravity-feed system. Unless otherwise stated, all recordings were made at 20–24°C and at a holding potential of −60 mV. Drugs were obtained from the Sigma Chemical Company. Most experiments were made on oocytes treated with collagenase to remove follicular and other enveloping cells (Miledi and Parker, 1984).

Results

Potentiation of GABA responses by barbiturate

Application of GABA to oocytes injected with rat or chick brain mRNA elicited smooth inward membrane currents (at −60 mV), which are due to the opening of membrane channels permeable mainly to chloride ions (Gundersen et al., 1984c; Miledi et al., 1982). Control (noninjected) oocytes showed either no responses to GABA or very small ones (a few nA) at millimolar concentrations of GABA. The size of the responses in injected oocytes was greatly potentiated when GABA was applied together with pentobarbital—Figure 1 (Gundersen et al., 1984a; Smart et al., 1983), even though pentobarbital, by itself, elicited very small responses, if any (see later). The onset of this potentiating effect was rapid. For example, the oocyte illustrated in Figure 1*B* showed a maximal potentiation within less than 50 sec of adding pentobarbital and recovered to the control value within a similar time after washing out the pentobarbital. The action of pentobarbital was probably even faster than this, since the exchange time of the perfusion chamber would have been the limiting factor.

Phenobarbital potentiated the responses to GABA in a manner similar to pentobarbital, but we did not examine this drug in detail.

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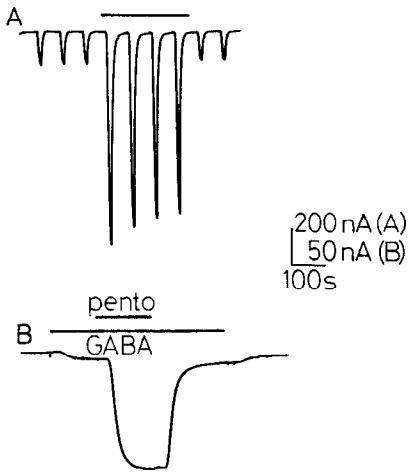

Figure 1. Potentiation of GABA-evoked membrane currents by pentobarbital. Records are from 2 collagenase-treated oocytes injected with rat brain mRNA. Oocytes were voltage-clamped at −60 mV, and the traces show clamp current. Inward membrane currents correspond to *downward deflections* in this and other figures. *A,* Responses evoked by 3 sec "puffs" of GABA (10^{-5} M) applied by bath perfusion at 60 sec intervals. During the time indicated by the *bar,* the GABA solution also contained 100 μM pentobarbital, but the oocyte was continuously perfused with normal Ringer's except for the brief puffs of drug solution. The decline in responses throughout the record probably arose from changes in the amount of drug delivered. *B,* Oocyte was perfused with GABA (10^{-6} M) for the duration indicated by the *lower bar,* and pentobarbital (100 μM) was added to this solution as indicated by the *upper bar.*

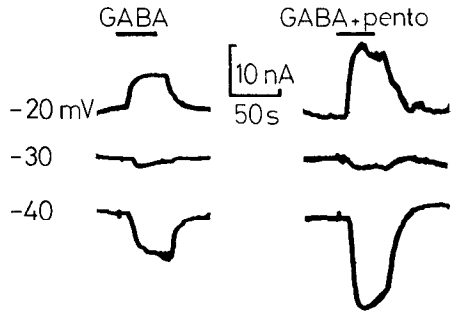

Figure 2. Reversal potential of the GABA-activated current. Traces show membrane currents in response to 10^{-5} M GABA applied as indicated by the *bars* and with the membrane potential clamped at the different levels shown. Records were obtained in normal Ringer's (*A*) and with 100 μM pentobarbital present in the GABA and wash solutions (*B*). Data from a single oocyte injected with rat brain mRNA.

Voltage dependence of the GABA-activated current

The membrane current activated by GABA decreased in size as the oocyte was depolarized and inverted direction at about −25 mV (Figs. 2*A,* 3), which corresponds to the chloride equilibrium potential in *Xenopus* oocytes (Barish, 1983; Kusano et al., 1982). This reversal potential is similar to that of GABA-activated channels induced in the oocyte by chick optic lobe mRNA (Miledi et al., 1982) and also to the chloride channels activated by ACh (muscarinic receptors), 5-HT, and glycine (Gundersen et al., 1983a, 1984a, c; Kusano et al., 1982). As the potential was made more negative, the peak GABA current increased to a maximum at about −75 mV but did not increase further with hyperpolarization beyond this voltage (Fig. 3). This rectification at negative potentials is similar to that seen with chloride currents activated by ACh, 5-HT, glutamate, glycine, and GABA (Gundersen et al., 1983a, 1984a, c; Kusano et al., 1982).

The reversal potential of the response to GABA was virtually unchanged in the presence of pentobarbital, even though larger currents were obtained at potentials away from the equilibrium (Figs. 2*B,* 3). The rectification at negative potentials was also still present (Fig. 3). Thus, the potentiation of the GABA response by pentobarbital seen at −60 mV does not arise from any shift in equilibrium potential or change in form of the current–voltage relationship. Instead, the degree of potentiation was similar at all potentials examined.

GABA dose–response curves

The size of the membrane current elicited by GABA was graded with the concentration of GABA, and at low doses, a doubling in concentration gave more than a 2-fold increase in response—Figure 4, *A, B* (see Miledi et al., 1982). Responses first became detectable at concentrations of between 2×10^{-7} and 10^{-6} M GABA, reaching a maximum at concentrations above about 10^{-4} M. At doses below about 10^{-5} M, the responses were well maintained for many seconds of drug application, but as the concentration was raised, the current declined increasingly rapidly (Fig. 4, *D, E*).

When GABA was applied together with pentobarbital (100 μM), responses became detectable at much lower GABA concentrations. For example, the oocyte in Figure 4 gave responses of similar size to 10^{-6} M GABA applied alone and to 5×10^{-8} M GABA together with 100 μM pentobarbital. However, the maximal response elicited at high concentrations of GABA was

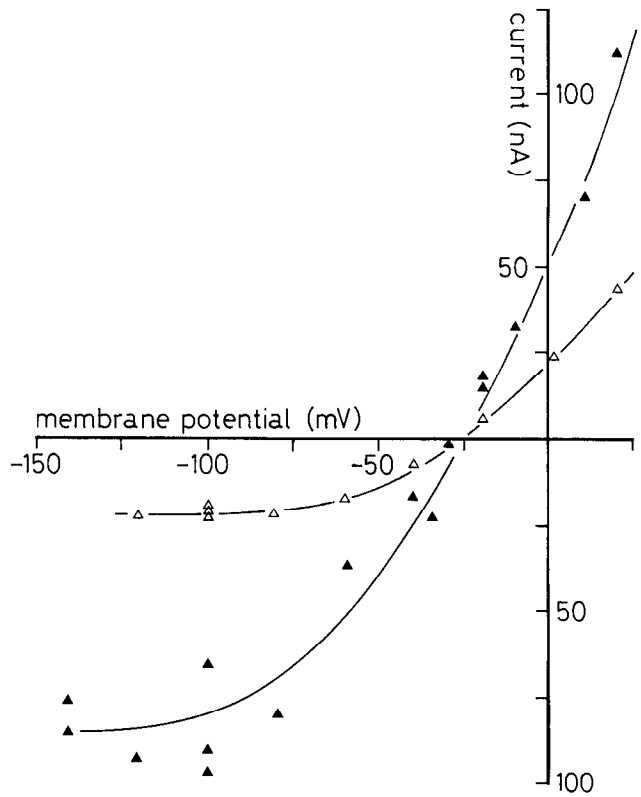

Figure 3. Current–voltage relationship of the currents activated by GABA (△) and GABA plus pentobarbital (▲). *Points* indicate peak currents elicited by 10^{-5} M GABA applied with the membrane potential clamped to different levels. Pentobarbital was at a concentration of 100 μM and was applied in both GABA and wash solutions. Data from the same oocyte as Figure 2.

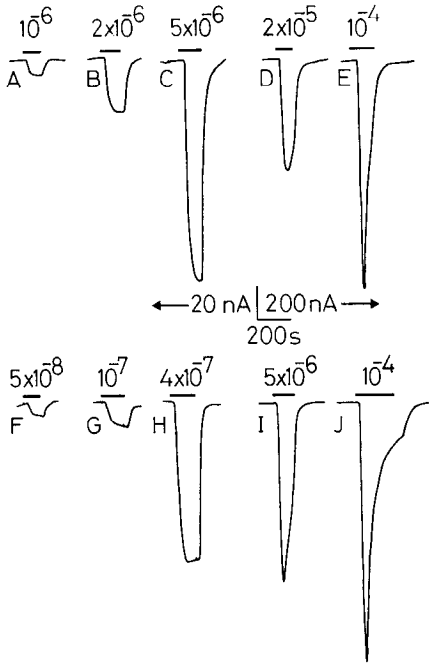

Figure 4. Responses elicited in a rat brain mRNA-injected oocyte by different concentrations of GABA. *A–E*, GABA alone; *F–J*, recordings in the continued presence of pentobarbital (100 μM). GABA was applied at the concentrations indicated (mM) for the times shown by the *bars*. Clamp potential was −60 mV. Note that the recording gain was 20 nA for traces to the *left* of the calibration bar (*A–C, F–H*), and 200 nA for those to the *right* (*D, E, I, J*).

little changed by pentobarbital, and for the oocyte illustrated, the response to 10^{-4} M GABA increased by less than 20% (Fig. 4, *E, J*).

Figure 5 shows dose–response curves, plotted on double-logarithmic coordinates, for GABA alone (open symbols) and GABA plus 100 μM pentobarbital (filled symbols). The data for GABA alone at concentrations below about 2×10^{-5} M lie on a straight line with a slope of 1.4, on the double-logarithmic coordinates. At higher concentrations, the increments in response with increases in concentration declined, and 10^{-4} M GABA gave a nearly maximal response. A further increase in the concentration from 10^{-3} to 10^{-2} M caused a slight decrease in the response. However, this may have arisen because of desensitization during the relatively slow drug application. For this reason also, the response sizes at concentrations above 10^{-5} M may have been underestimated.

When pentobarbital (100 μM) was applied together with GABA, the dose–response curve for GABA was shifted to the left by roughly a decade (Fig. 5). Half-maximal activation of the response required a concentration of about 3×10^{-5} M GABA alone, but only about 4×10^{-6} M GABA in the presence of 100 μM pentobarbital. The maximal response at high concentrations (10^{-3} M and greater) of GABA was, on average, little changed by pentobarbital, some oocytes showing a small increase and others a small decrease. In 5 oocytes the mean response to 10^{-3} M GABA plus 100 μM pentobarbital was 92% (±12% SEM) of the response to the same concentration of GABA alone.

In addition to the leftward shift of the dose–response relationship, a further action of pentobarbital was to alter the limiting slope of the dose–response relationship at low concentrations, so that the response size varied almost linearly with the concentration of GABA (slope of 1.15 on double-logarithmic coordinates with 100 μM pentobarbital).

Desensitization of GABA responses

Desensitization of the GABA responses to low GABA concentrations became prominent when the currents were potentiated by pentobarbital. For example, the oocyte of Figure 4 showed little desensitization during application of 5×10^{-6} M GABA, but it exhibited a marked decline in responsiveness with the same GABA concentration in the presence of 100 μM pentobarbital (cf. Fig. 4, *C, I*).

To quantify this effect, we measured the percentage by which the current had declined 50 sec after the peak. Responses to GABA at concentrations below about 10^{-5} M showed virtually no fall at this time, but a decline became evident as the concentration was raised, reaching a maximal value of about 65% at concentrations of 10^{-3} M and above (Fig. 6*A*, open symbols).

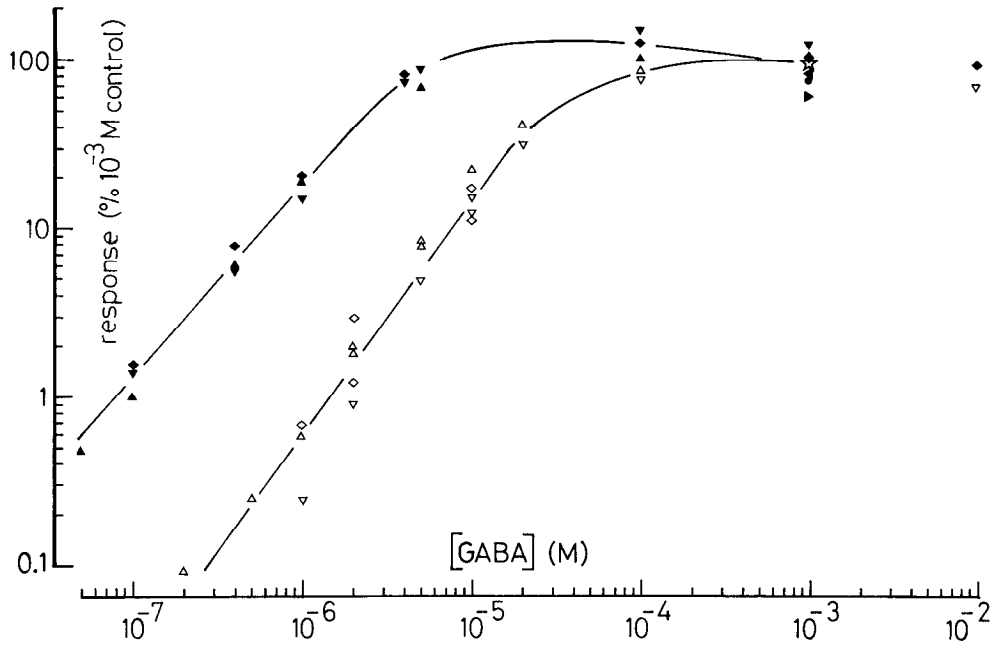

Figure 5. Dose–response curves for GABA alone (*open symbols*) and GABA plus 100 μM pentobarbital (*filled symbols*) plotted on double-logarithmic coordinates. Data were obtained from records similar to those in Figure 4 and are normalized as a fraction of the response in each oocyte to 10^{-3} M GABA applied alone (*star*). Clamp potential was −60 mV. Measurements from 5 oocytes (*different symbols*) that gave responses to 10^{-3} M GABA between 420 and 1600 nA; *upright triangles*, data from the same oocyte as Figure 4.

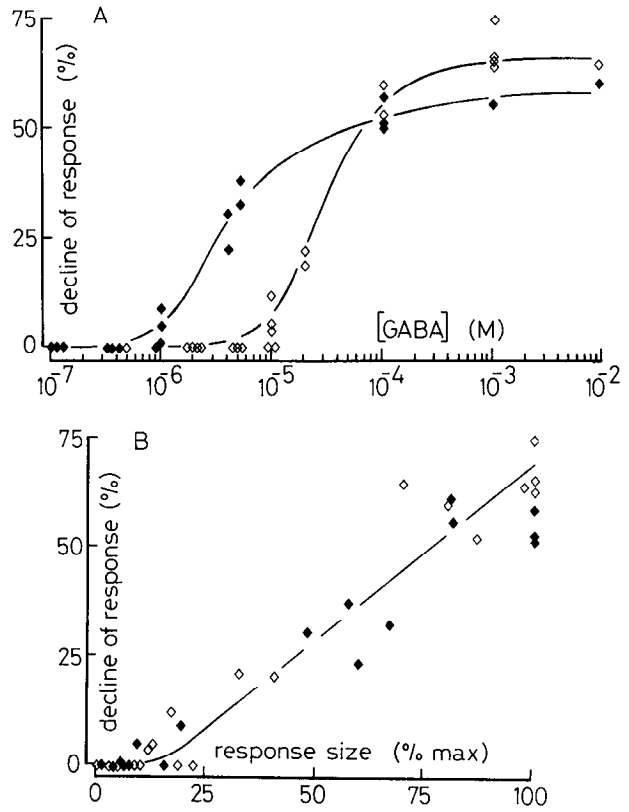

Figure 6. Desensitization of the GABA-activated current. In both graphs, the vertical axis indicates the decline of the response measured 50 sec after the peak, expressed as a percentage of the peak response. *Open symbols,* Measurements with GABA alone; *filled symbols,* GABA plus 100 μM pentobarbital. *A,* Data from 3 oocytes (the same as in Fig. 5) plotted showing desensitization as a function of concentration of GABA. *B,* Same data as *A,* but with desensitization plotted against the normalized peak size of the response.

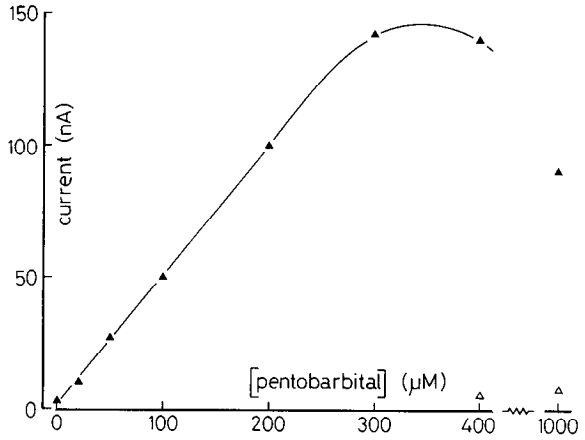

Figure 7. Potentiation of GABA response by different concentrations of pentobarbital. Measurements were made by exposing the oocyte to each concentration of pentobarbital for a few minutes and then recording the peak response to 2×10^{-6} M GABA in the continued presence of pentobarbital. *Filled symbols,* Peak GABA response; *open symbols,* current elicited by pentobarbital alone. Data from a single oocyte clamped at −60 mV.

In the presence of pentobarbital, this relationship was shifted leftward along the concentration axis by about a decade (Fig. 6*A*, filled symbols), so that desensitization was first observed at a concentration of about 10^{-6} M GABA and peaked at about 10^{-4} M. The maximal rate of decline of the response at high concentrations of GABA was also slightly smaller in the presence of pentobarbital.

Figure 6*B* shows the percentage decline of the response, 50 sec after the peak, plotted against the peak response size. The data for GABA alone (open symbols) and GABA plus 100 μM pentobarbital (filled symbols) closely follow the same relationship. Thus, the rate of desensitization of a response potentiated by pentobarbital matched that of a response of similar size elicited by a higher concentration of GABA applied alone. Responses of less than about 10% maximal (elicited by GABA or by GABA plus pentobarbital) showed no obvious desensitization 50 sec after the peak, but with larger responses the extent of the desensitization rose as a roughly linear function of the peak size.

Although the rate of desensitization was related to the peak size of the response, it was not determined by the magnitude of the current flow *per se,* since for a given dose of GABA, the extent of the decline remained almost unaltered over a range of clamp potentials. For example, the time to half-decay of the response elicited by 1 mM GABA was about the same at clamp potentials of −26 and −60 mV, even though the peak current was about 10 times smaller at the lower potential.

Potentiation depends on pentobarbital concentration

The degree of potentiation of the GABA response produced by different concentrations of pentobarbital was examined by exposing oocytes to a fixed dose of GABA together with different concentrations of pentobarbital. A low concentration of GABA was chosen, so that even with large potentiations the response size remained smaller than the maximum that could be elicited by high GABA concentrations.

As the concentration of pentobarbital was raised from 0 to about 300 μM, the GABA-activated response increased linearly. Raising the concentration further to 1 mM caused a fall in the response (Fig. 7). At high pentobarbital concentrations (>400 μM), the membrane current increased transiently when the GABA plus barbiturate solution was replaced by normal Ringer's, whereas at low concentrations the response declined smoothly during washing.

The potentiation obtained with 300–400 μM pentobarbital was very large, and for the oocyte illustrated, the response was 50 times greater than with the same concentration of GABA (2×10^{-6} M) applied alone.

Voltage-jump relaxations

In cultured mouse spinal motoneurons, noise analysis has shown that phenobarbital prolongs the opening of single GABA-activated channels (Barker and McBurney, 1979). To determine whether pentobarbital had similar effects on the rat brain GABA channel expressed in the oocyte, we examined the relaxation of the clamp currents following step changes in membrane potential during steady application of GABA or GABA plus pentobarbital. This technique has been used to study ACh channels in muscle (Adams, 1977; Neher and Sakmann, 1975), where the current was found to relax exponentially to a new steady level with a time constant corresponding to the mean channel lifetime at the potential following the step.

Hyperpolarizing voltage steps, from a holding potential of −40 mV, elicited almost rectangular current steps in the absence of drugs (Gundersen et al., 1984c; Miledi, 1982). However, the same potential steps applied in the presence of GABA gave an initially larger current, followed by a rapid decline to a new steady level. The upper of the 2 superimposed traces in Figure 8, *A, B,* illustrates the relaxation of the GABA-activated current during hyperpolarizing steps, after subtraction of the passive

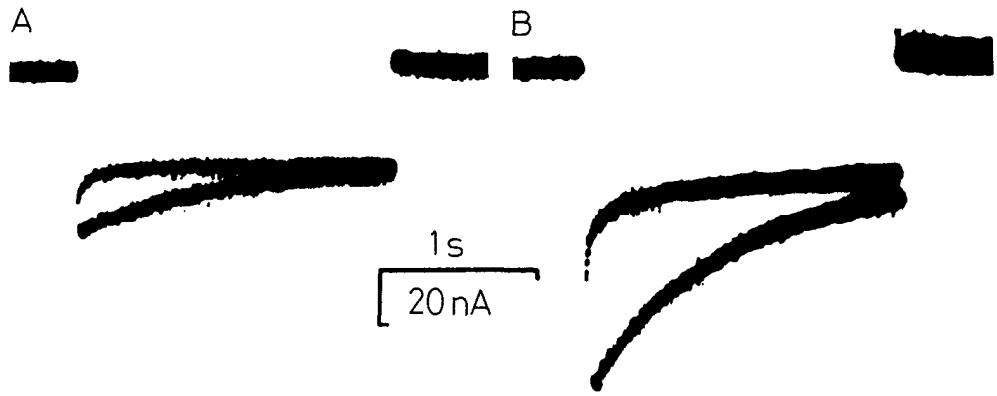

Figure 8. GABA-activated current relaxations following voltage steps. The oocyte membrane was clamped at a potential of −40 mV and briefly displaced to −80 mV (*A*) or −140 mV (*B*). Responses to voltage steps were recorded before and during drug application, and the traces show records of the drug-activated current after subtraction of the passive currents elicited in the resting oocyte. Each frame shows 2 superimposed recordings, the *upper traces* obtained with 10^{-5} M GABA and the *lower traces* with 2×10^{-6} M GABA plus 100 μM pentobarbital. Responses of roughly equal size (ca. 55 nA at −60 mV) were elicited by these concentrations of GABA and GABA plus pentobarbital.

membrane currents. The current relaxation appeared to follow 2 exponential components, the first with a time constant of less than 10 msec and the second with a time constant of 140 msec at −80 mV and 220 msec at −140 mV. In the presence of pentobarbital (100 μM), the relaxation of the GABA-activated currents was much slower than with GABA alone (Fig. 8, *A, B,* lower traces). The decline of the currents closely followed a single-exponential relationship, and for the oocyte illustrated, the time constant of the decline at −80 and −140 mV was about 750 msec. Records from 2 other oocytes gave similar values.

Pentobarbital directly activates a membrane current

Application of pentobarbital to rat brain mRNA-injected oocytes elicited, by itself, inward membrane currents at a membrane potential of −60 mV. These responses were usually only a few nanoamperes with a concentration of 100 μM pentobarbital and were generally negligible compared with the potentiated GABA responses. However, at high concentrations, the pentobarbital current became more prominent (Fig. 7, open symbols).

Large responses to pentobarbital were also obtained in oocytes

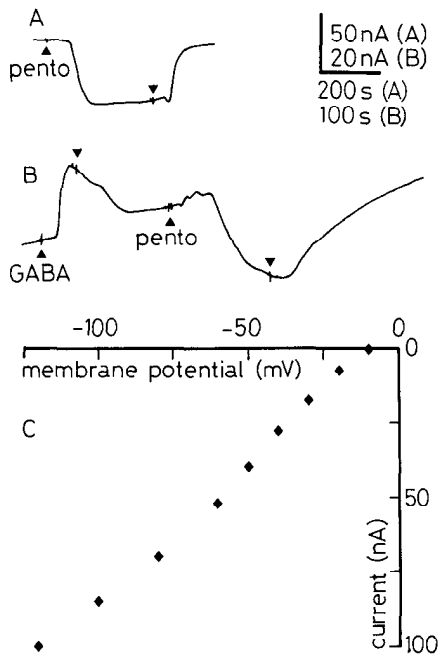

Figure 9. Membrane currents elicited by pentobarbital in oocytes injected with mRNA from chick optic lobe. *A,* Maintained inward current elicited by 10^{-3} M pentobarbital applied as indicated (*arrowheads*) with the oocyte clamped at −60 mV. *B,* Records from a different oocyte soon after clamping at −12 mV show the outward current elicited by GABA (5×10^{-5} M) and the inward current elicited by pentobarbital (10^{-3} M). *C,* Current–voltage relationship for the current elicited by 10^{-3} M pentobarbital. Data were obtained by briefly stepping the potential to different levels during sustained application of pentobarbital and are plotted after subtraction of "passive" currents in the absence of drug. Same oocyte as in *A.*

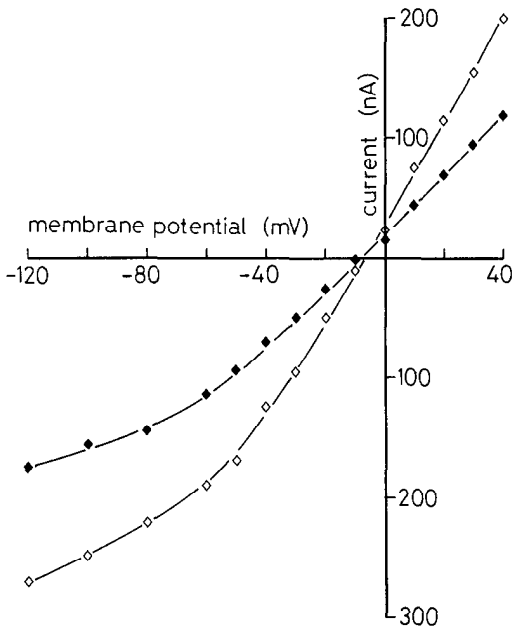

Figure 10. Pentobarbital depresses the response to kainate, without altering the reversal potential or the shape of the current–voltage relationship for 5×10^{-5} M kainate (□) and the same concentration of kainate plus 10^{-4} M pentobarbital (■). Measurements were made by applying voltage steps during the maintained response to kainate and are plotted after subtraction of "passive" membrane currents (as in Fig. 3). Data from a single oocyte injected with rat brain mRNA.

injected with mRNA derived from chick optic lobe (Miledi et al., 1982). The current elicited by 1 mM pentobarbital in the chick mRNA-injected oocytes was well maintained during drug application (Fig. 9*A*), and voltage steps applied during the response evoked increased membrane currents, indicating that pentobarbital caused an increase in membrane conductance. As the membrane potential was made less negative, the response decreased in size, declining to zero at a potential of about −10 mV (Fig. 9*C*). This value was more positive than the equilibrium potential for the GABA-activated current, and Figure 9*B* clearly shows that the 2 currents invert at different potentials when measured in the same oocyte. At a clamp potential of −12 mV, GABA elicited an outward membrane current, while the predominant current elicited by pentobarbital was inward. We do not yet know the ionic basis of the response to pentobarbital, but it may comprise 2 different components, since at −12 mV the inward current was preceded by a smaller outward component (Fig. 9*B*). At potentials negative to the equilibrium, the main pentobarbital-evoked current showed an almost linear voltage dependence (Fig. 9*C*).

Other actions of pentobarbital

In addition to the potentiation of GABA responses, pentobarbital affects other drug- and voltage-activated currents in the oocyte. One of these is the transient outward (T_{out}) current, which is activated by depolarization. During application of 1 mM pentobarbital, the T_{out} current was reduced to about one-quarter but recovered quickly after washing. T_{out} is carried by chloride ions, and depends on an influx of calcium through voltage-gated channels (Barish, 1983; Miledi, 1982; Miledi and Parker, 1984). It remains to be seen whether pentobarbital reduced the calcium influx or blocked the calcium-activated chloride channels.

A further action of pentobarbital was to reduce the currents elicited by activation of kainate receptors induced in rat brain mRNA-injected oocytes (Gundersen et al., 1984a). At a concentration of 100 μM, pentobarbital diminished the response to kainate by about 60%, but did not alter the equilibrium potential or the form of the current–voltage relationship (Fig. 10). The oscillatory chloride currents elicited by glutamate in a rat brain mRNA-injected oocyte (Gundersen et al., 1984a) were also reduced by pentobarbital.

In marked contrast to the potentiation of the GABA responses, pentobarbital failed to enhance the chloride currents elicited by activation of glycine receptors translated by rat brain mRNA (see also Gundersen et al., 1984c). In 3 oocytes examined, the mean size of the response to glycine (10^{-5} M) plus pentobarbital (100 μM) was 98% of that to the same concentration of glycine alone, even though the responses to GABA recorded in the same oocytes were strongly potentiated.

Discussion

We have so far found barbiturates to have 5 major actions on rat brain membrane channels transplanted to the *Xenopus* oocyte: (1) potentiation of responses elicited by GABA; (2) potentiation of responses to β-alanine, probably involving a specific receptor type (I. Parker, K. Sumikawa, and R. Miledi, unpublished observations); (3) reduction of responses to kainic acid; (4) direct activation of membrane channels; and (5) reduction of the calcium-dependent chloride transient outward current. Most of these effects have been observed in "native" neuronal cells in the CNS (Akaike et al., 1985; Barker and Ransom, 1978; Willow and Johnston, 1983). It appears, therefore, that the receptor-channel complexes translated by the rat brain mRNA have properties similar to those of native neurons. However, the study of barbiturate action becomes much easier when the receptors are expressed in the oocyte. In particular, known concentrations of drugs can be applied without the uncertainties of iontophoretic or microperfusion techniques, and without the complications arising from active uptake of GABA from the synaptic cleft. Against this, the oocyte technique does introduce some new problems—including the influence of a foreign lipid environment on the receptor-channel functioning and the possibility that more than a single type of GABA receptor may be expressed.

Actions of pentobarbital on GABA responses

A major effect of pentobarbital on the responses elicited by GABA in oocytes injected with rat brain mRNA was to increase the apparent affinity of the receptor for GABA, without substantially changing the maximal response at high concentrations of GABA. The extent to which the GABA response was potentiated increased with the dose of pentobarbital and followed a linear relationship for concentrations up to about 300 μM. This suggests that the modulation of the GABA response by pentobarbital may involve the binding of only 1 molecule of barbiturate to the GABA receptor-channel complex. The greatest potentiation we observed was about a 50-fold (with 2×10^{-6} M GABA and 300 μM pentobarbital); from the observed dose–response relationship (Fig. 5), this would correspond to an increase in apparent affinity of the receptor for GABA of about 20 times.

When the pentobarbital concentration was raised above about 300 μM, the current activated by a constant dose of GABA began to decline. The reason for this is not clear. Possibilities include a plugging of the chloride channels by high concentrations of barbiturate, similar to that seen at ACh-activated endplate channels (Adams, 1976); occupation of the GABA receptor sites; or reversal of the enhancement of GABA binding (Willow and Johnston, 1980). Whatever its origin, the depression at high barbiturate concentrations was rapidly reversible, since the responses often increased transiently, before falling, when GABA and barbiturates were washed from the oocyte. A similar decline in potentiation of GABA responses at high (200 μM–1 mM) concentrations of pentobarbital has been reported in the rat dorsal root ganglion (Connors, 1980) and cuneate nucleus (Simmonds, 1981).

At low GABA doses, the size of the evoked current increased more than linearly with concentration, following a 1.4 power relation. In the presence of 100 μM pentobarbital, this relationship was shifted to the left by roughly a decade. This result is similar to that observed for GABA receptors in the rat cuneate nucleus (Simmonds, 1981), where the same concentration of pentobarbital gave a 7-fold parallel shift. However, the GABA dose–response curve in the oocyte was not simply displaced in a parallel manner by pentobarbital; instead, the concentration dependence became close to linear (Hill coefficient of 1.15 with 100 μM pentobarbital). Because of the change in limiting-slope value on a double-logarithmic dose–response plot, the pentobarbital potentiation varied with the dose of GABA (even at concentrations giving well below maximal activation) and became proportionally greater at lower concentrations. These results suggest that 2 or more molecules of GABA are required to cause the opening of the chloride channel when GABA is applied alone (see also Akaike et al., 1985). However, this cooperativity is reduced by pentobarbital, so that perhaps binding of only 1 molecule of GABA to the receptor then becomes sufficient to trigger the opening of the channel.

Half-maximal activation of the GABA current was obtained at a concentration of about 30 μM, which is similar to the value of the apparent dissociation constant of 100 μM estimated by patch-clamp recording of GABA channels in rat hippocampal neurons (Sakmann et al., 1983). This is, however, still more than 10 times greater than the apparent dissociation constant of the "low affinity" GABA receptors derived from radioligand binding measurements (see Olsen, 1982, for review). Compar-

ison of affinities obtained from electrophysiological and binding measurements is complicated by the use of rather unphysiological conditions in the binding studies, but the large discrepancy suggests that the GABA binding sites in those assays may not correspond with the sites that mediate functional activity.

Relaxations of GABA-activated currents following voltage steps were slowed in the presence of pentobarbital, suggesting that a major action of pentobarbital may be to prolong the mean open time of the GABA-activated channel in the oocyte membrane. This is in agreement with studies on mammalian central neurons, where the open time of the GABA channel, estimated by noise analysis (Study and Barker, 1981) and decay of inhibitory postsynaptic currents (Collingridge et al., 1984), was found to be prolonged. The voltage-jump relaxations were slowed by a factor of about 4 by a concentration of pentobarbital (100 μM) that gave a roughly 10-fold increase in steady-state current. It is therefore unclear whether this effect can account entirely for the potentiation. The presence of 2 components in the relaxation of the GABA-activated current and the decrease in voltage sensitivity of the relaxation seen with pentobarbital complicate the interpretation of the voltage-jump experiments, and more work will be needed to determine the mechanisms involved.

Desensitization of GABA responses

In the presence of pentobarbital, GABA-activated currents desensitized more rapidly than with the same concentrations of GABA applied alone. However, the rate of desensitization was comparable for similarly sized responses, whether produced by GABA plus pentobarbital or by a higher concentration of GABA alone. Thus, the desensitization of the GABA response appears to depend on the extent of functional activation of the channels, rather than on the absolute concentration of the agonists. Furthermore, the rate of desensitization was found to be unchanged when the GABA-activated current was altered by changing the membrane potential, indicating that desensitization does not depend on the current flow *per se,* but instead is probably determined by the frequency of channel openings.

Responses to GABA recorded from neurons in the CNS also show desensitization, but in this case it has been suggested that active uptake of GABA may be at least partially responsible for the decline in response (Snodgrass, 1983). Our results indicate, however, that desensitization of the brain GABA receptor itself is an important mechanism in determing the response to prolonged GABA application. Although we do not yet know whether the oocyte possesses any "native" uptake system for GABA or whether uptake is induced following the injection of brain mRNA, it is unlikely that the concentration of GABA in the continuously flowing solution next to the oocyte would be appreciably reduced even if uptake into the oocyte did occur. This is especially so because the experiments were made on oocytes that had been treated with collagenase to remove follicular and other enveloping cells.

Pentobarbital currents

Pentobarbital, alone, elicited membrane currents in oocytes injected with rat and chick brain mRNA. These responses were accompanied by an increase in membrane conductance and inverted at about −10 mV, a potential that was clearly different from the reversal of the chloride currents activated by GABA. A part of the response may have arisen from activation of a chloride conductance, but it is clear that there was also a current with a more positive equilibrium potential. This component of the pentobarbital current in the oocyte appears to differ from those responses previously seen in neurons, which were due either to an increase in chloride conductance (Akaike et al., 1985; Barker and Ransom, 1978; Nicoll, 1975) or to a decrease in potassium conductance (Higashi and Nishi, 1982).

Clinical effects

Pentobarbital and related drugs probably exert their clinical effects through several combined actions—including enhancement of inhibitory amino acid transmission, depression of excitatory amino acid transmission, and direct actions on membrane channels. Inhibitory synaptic currents in the brain presumably result from a brief, high concentration of transmitter released into the synaptic cleft. Our results suggest that under these conditions, the main effect of pentobarbital would be to prolong the duration of the synaptic current, rather than to increase its size; and, indeed, this is what is observed (Collingridge et al., 1984). However, if there is a steady "leakage" of GABA from inhibitory nerve terminals, similar to that of ACh at the motor endplate (Katz and Miledi, 1977), then the large potentiating effect of pentobarbital at low doses of GABA could have significant additional effects on neuronal excitability.

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