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# Effects of some depressant drugs on synaptic responses to glutamate at the crayfish neuromuscular junction

Ruth E. Wachtel[1]

Nerve-Muscle Research Centre, School of Physiology & Pharmacology, University of New South Wales, Kensington, N.S.W. 2033, Australia

**1** Excitatory junction currents produced by glutamate were recorded with an extracellular electrode at the neuromuscular junction of the crayfish.

**2** Pentobarbitone, phenobarbitone, diazepam, chlordiazepoxide and procaine had only minimal effects on current decay at concentrations which are highly effective in other preparations. The glutamate synapse in the crayfish appears relatively resistant to these drugs.

**3** In contrast, ether and halothane increased the rate of decay of the currents at concentrations which are comparable to those occurring during anaesthesia.

## Introduction

A number of drugs which are depressants of the nervous system may exert their effects through alterations in synaptic transmission. Sedatives, anticonvulsants and anaesthetics have all been shown to depress excitatory postsynaptic responses to neurotransmitters in different preparations (see reviews by Richards, 1978; 1980).

At cholinergic synapses, these compounds often alter postsynaptic potentials by modifying the decay phase of postsynaptic currents (see review by Gage & Hamill, 1981). For example, barbiturates, diazepam and volatile general anaesthetics all increase the rate of decay of currents at the motor endplate of mammals and amphibia (Gage & Hamill, 1975; 1976; Adams, 1976; Torda & Gage, 1977; Gage *et al.*, 1979). In the presence of local anaesthetics, miniature endplate currents produced by acetylcholine decay with a characteristic biphasic, or double exponential, time course.

Although glutamate is generally considered to be an excitatory transmitter in the mammalian central nervous system, the pharmacology of glutamate synapses has not been studied extensively. Anaesthetic compounds depress depolarizing responses to applied glutamate (Richards & Smaje, 1976), but their effects on glutamate-activated currents are not known.

In order to determine the effects of some anaesthetic compounds on responses to glutamate, postsynaptic currents were measured at the excitatory neuromuscular junction of the crayfish, where glutamate is the transmitter. Excitatory junction currents (e.j.cs) produced by nerve stimulation, representing an increase in permeability mainly to $Na^+$ (Dudel, 1974; Onodera & Takeuchi, 1975), were recorded with an extracellular electrode.

The decay phase of e.j.cs was shortened by the volatile general anaesthetics ether and halothane, and this effect occurred at concentrations which are comparable to those found clinically. In contrast, barbiturates, benzodiazepines and procaine had very little effect on the time course of excitatory currents in the crayfish.

## Methods

Experiments were performed on the opener muscle (abductor) of the dactyl of the first 2 walking legs of Australian freshwater crayfish, or yabbies (*Cherax destructor*). Animals averaging 6–8 cm in length were obtained from Narrandera, N.S.W., and were kept for up to 4 months before use.

Legs were amputated at the autotomy joint and the fixed claw and adjoining propodite were cut away. The entire closer muscle and also the bundles of sensory fibres lying between the closer and opener muscles were carefully removed to expose the opener. The preparation was immobilized by inserting it between rows of insect pins in a chamber lined with black Sylgard (Dow Chemical Co.), and was continuously perfused with a solution containing (mM): NaCl 205, KCl 5.4, $CaCl_2$ 13.5, $MgCl_2$ 2.6,

[1]Present address: Department of Anesthesia, University of Iowa, Iowa City, Iowa 52242, U.S.A.

and HEPES buffer (N - 2 - hydroxyethylpiperazine - N' - 2 - ethane sulphonic acid) 5, adjusted to pH 7.5. The muscle was illuminated from above using a fibre optics light guide. Temperature was controlled with a Peltier cooling device, and was usually adjusted to 11–14°C.

The nerve innervating the walking leg was dissected free in the meropodite by removing surrounding exoskeleton and muscle, and was separated along its natural cleavage into thick and thin bundles. The thick bundle, which contains both the excitatory and inhibitory axons, was stimulated with a suction electrode, while an extracellular electrode was moved over the nerve until 2 extracellular action potentials could be recorded from the same site. The nerve bundle was then separated into smaller divisions until the excitatory axon could be stimulated independently of the inhibitory one.

The excitatory axon was then selectively stimulated and the extracellular electrode was placed at several points on the surface of muscle fibres until a negative deflection, corresponding to an inward current produced by the action of glutamate on the postsynaptic membrane, could be detected. The position of the extracellular electrode was carefully adjusted to ensure that it was directly over a synaptic region and not recording field potentials due to the activity of synapses which were merely nearby the electrode. Recordings were acceptable only if the rise time of the currents was rapid (250–300 μs) and if there was no deflection of the baseline when the nerve terminal failed to release transmitter. The stimulation rate was then adjusted to between 2 and 8 Hz to produce a high failure rate in order to minimize the effects of transmitter release at nearby synapses. Evoked responses were usually produced by the release of only 1–2 quanta of transmitter. Spontaneous synaptic currents were sometimes recorded, although their frequency was often very low ($<1$ per min). Spontaneous currents were analysed together with evoked responses.

Extracellular electrodes were filled with the normal perfusing solution, and had resistances of 2 MΩ. Tip diameters ranged from 5 to 20 μm. For intracellular recording, electrodes were filled with 2.5 M KCl and had resistances of about 5 MΩ.

The following drugs were applied by bath perfusion: diazepam, chlordiazepoxide, procaine (courtesy Australian Pharmaceutical Industries), pentobarbitone and phenobarbitone (courtesy Prosana Laboratories), and halothane (Fluothane, ICI Australia). All were applied at pH 7.5, except for the barbiturates, which were applied at their $pK_a$: 8.0 for pentobarbitone and 7.3 for phenobarbitone. Diazepam and chlordiazepoxide were dissolved by lowering the pH.

Extracellular currents (e.j.cs) were high pass filtered at 1 Hz and low pass filtered at 4 kHz with a 4-pole Bessel filter. Data were digitized online at 56 μs per point by a 6809-based microcomputer and stored on floppy disks. Records were edited to eliminate currents which contained an abrupt change in slope in either the rising or falling phases, probably due to the asynchronous release of more than one quantum of transmitter, or which had rise times much greater than 300 μs. Currents (usually 20–100) were aligned at their peaks before averaging.

The decay phase of both individual and averaged e.j.cs was a complex function of time, and was not exponential. In order to obtain a quantitative estimate of the time course of the currents, the decay phase was described by a $t_{1/2}$, or the time required to decay to one-half the peak height, and also by $A_I$ the area under a normalized peak. The area $A_I$ was calculated by summing the digitized points of the averaged e.j.c., then dividing by the peak height in order to eliminate the effects of variations in seal resistance between the muscle membrane and the extracellular electrode. Thus $A_I$ is in units of time (ms). Measurements of $t_{1/2}$ and $A_I$ obtained in the presence of the drugs are expressed as a percentage of control values.

## Results

The data are summarized in Table 1.

### *Volatile general anaesthetics*

Halothane greatly increased the rate of decay of glutamate-activated postsynaptic currents and both $t_{1/2}$ and $A_I$ were reduced in a dose-dependent manner. In the experiment shown in Figure 1, 1.0 mM halothane decreased $t_{1/2}$ and $A_I$ to an average of 69% and 71% of control values, respectively. Lower concentrations had a smaller effect, while much higher concentrations were extremely effective in reducing the amplitude of the currents and increasing their rate of decay. With 20 mM halothane, individual currents were barely visible, and $t_{1/2}$ of the averaged e.j.c. was reduced to 0.4 ms. These effects were reversible, and $t_{1/2}$ and $A_I$ returned toward control values upon washing of the preparation. In 3 separate experiments, 1.0 mM halothane reduced both $t_{1/2}$ and $A_I$ to an average of 79% of control values.

Ether was also quite effective in increasing the decay rate of the currents (not shown). With 10 mM ether, both $t_{1/2}$ and $A_I$ were reduced to 78% of control values (mean of 4 experiments). With 70 mM ether, $t_{1/2}$ and $A_I$ were decreased to an average of 49% and 58%, respectively.

During anaesthesia, serum levels of halothane are probably about 0.2 mM (Gage & Hamill, 1976). Although only small effects were observed below

**Table 1** Summary of drug effects on the decay phase of postsynaptic responses to glutamate

| *Drug* | *Concentration* (mM) | $t_{1/2}$ (*% of control*) | $A_I$ (*% of control*) |
|---|---|---|---|
| Halothane | 1 | 69, 84, 85 (79) | 71, 82, 84 (79) |
| Ether | 10 | 66, 81, 83, 84 (78) | 64, 79, 84, 83 (78) |
| Pentobarbitone | 1 | 78, 80, 86 (81) | 78, 69, 77 (75) |
| Phenobarbitone | 1 | 88, 98 (93) | 86, 96 (91) |
| Diazepam | 0.1 | 104, 106, 119 (110) | 100, 107, 118 (108) |
| Chlordiazepoxide | 0.2 | 90, 92 (91 | 91, 96 (94) |
| Procaine | 0.5 | 100, 111, 112 (108) | 127, 116, 111 (118) |

$t_{1/2}$ is the time required for the e.j.c. to decay to one-half its peak height. $A_I$ represents the area under the decay phase of the current after it has been normalized to a standard peak height. All values are expressed relative to control. Each number is a separate experiment and the mean is given in parentheses.

0.5 mM halothane, the true concentration of halothane in the solution was probably less than expected due to the volatile nature of the anaesthetic. For ether, decreases in $t_{1/2}$ and $A_I$ were seen at concentrations identical to those found clinically: Gage & Hamill (1976) have estimated that the free concentration of ether in the blood during anaesthesia is 10–20 mM. Therefore, the observed increase in the rate of current decay may represent an important effect of ether during anaesthesia.

*Barbiturates*

Pentobarbitone also increased the rate of decay of the currents, although this effect was observed only at relatively high concentrations. At a concentration of

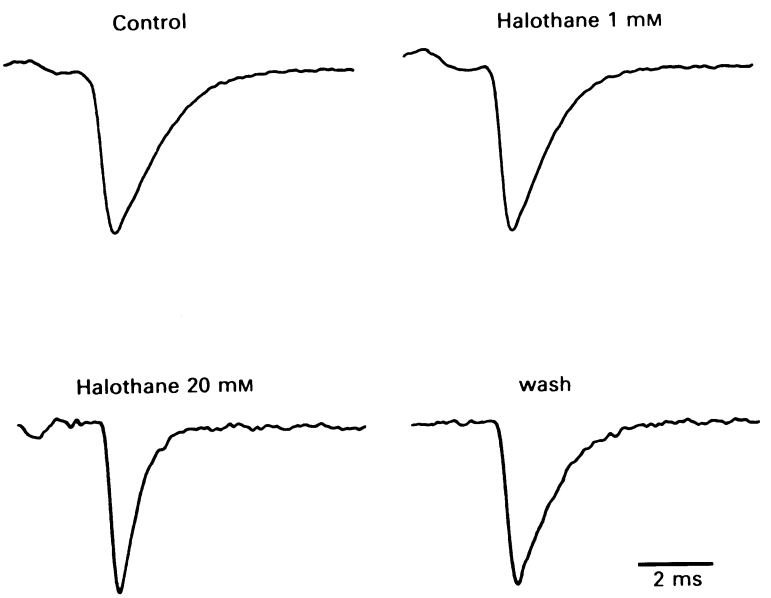


**Figure 1** Averaged excitatory junction currents recorded in the absence and presence of halothane. At 1.0 mM halothane, $t_{1/2}$ decreased by 31%, from 1.79 to 1.23 ms, and $A_I$ decreased by 29%, from 1.96 to 1.40. At 20 mM, the currents in another fibre were extremely small and decayed very rapidly; $t_{1/2}$ was reduced to 0.4 ms, and $A_I$ was reduced to 0.6. The effects of halothane were reversible upon washing. The deflection at the beginning of each trace is an extracellular action potential, which has been broadened during averaging due to variations in synaptic delay at this site. Note that currents have been normalized to the same peak height.

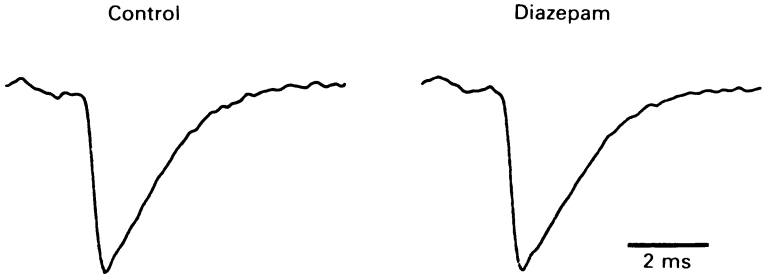


**Figure 2** Averaged excitatory junction currents recorded in the absence and presence of 0.1 mM diazepam. After the addition of diazepam, $t_{1/2}$ increased by 6%, from 1.85 to 1.96 ms, while $A_I$ increased by 7%, from 2.11 to 2.26.

1 mM, pentobarbitone produced an average decrease in $t_{1/2}$ and $A_I$ to 81% and 75% of control values (mean of 3 experiments). Lower concentrations of the drug produced smaller changes in current decay, while higher concentrations blocked action potential conduction.

In 2 experiments, phenobarbitone had only a small effect on the decay of the currents. At a concentration of 1.0 mM, $t_{1/2}$ and $A_I$ were reduced by only about 10%.

Since the effective blood level of phenobarbitone during anticonvulsant therapy is approximately 45–110 μM, of which 50% is bound to plasma proteins (Eadie, 1976), phenobarbitone would not seem to have any significant effect on the time course of glutamate currents at clinical concentrations. Similarly, the effects of pentobarbitone were relatively small, considering the high concentration. In other preparations, barbiturates often alter postsynaptic currents at concentrations of 100 μM or less (Adams, 1976; Torda & Gage, 1977; Wachtel & Wilson, 1983; Robertson & Gage, unpublished observations), and therefore it appears as if glutamate currents at the crayfish neuromuscular junction are fairly insensitive to the effects of the barbiturates.

*Benzodiazepines*

As shown in Figure 2, 0.1 mM diazepam produced a very slight lengthening of glutamate currents and a small increase in $t_{1/2}$ and $A_I$. In 3 experiments this increase averaged only about 10%. Lower concentrations (1–10 μM) had no effect on the currents, while higher concentrations (0.2–0.5 mM) often produced a larger increase in the duration of the responses. At 0.5 mM, a conduction block sometimes developed, and extracellular action potentials disappeared even though the axon was being maximally stimulated. Extracellular spikes and glutamate currents returned upon washing of the preparation. Chlordiazepoxide, at concentrations as high as 0.5 mM, produced only a small decrease in $t_{1/2}$ and $A_I$. At 0.2 mM, the decrease was no more than 10%.

The therapeutic serum level of chlordiazepoxide following a therapeutic dose is in the order of 5 μM (Gottschalk & Kaplan, 1972), which is certainly far below the levels tested in these experiments. The glutamate synapse in the crayfish seems to be relatively insensitive to the benzodiazepines.

*Procaine*

Although procaine is a depressant of the peripheral, rather than the central nervous system, and is in fact a convulsant when administered centrally, it was included in this study because it produces a characteristic biphasic decay of miniature endplate currents at cholinergic neuromuscular junctions. Procaine (0.5 mM) caused a small increase in $t_{1/2}$ and $A_I$ (Figure 3), but $A_I$ was increased slightly more than $t_{1/2}$ (118% vs. 108%). Procaine seemed to produce a small tail at the end of the currents. Higher concentrations of procaine (1 mM) usually blocked action potential conduction.

Procaine produces biphasic miniature endplate currents at the cholinergic neuromuscular junction at concentrations as low as 0.1 mM, while 0.5 mM has dramatic effects on the shape of the decay phase. Again, this glutamate synapse appears to be only minimally affected by the drug at concentrations which are highly effective in other systems.

## Discussion

Barbiturates and benzodiazepines have little effect on the decay of e.j.cs at concentrations which approximate therapeutic blood levels. Higher concentrations of these drugs had a small effect on the decay phase; 1.0 mM pentobarbitone decreased $t_{1/2}$, while 0.1 mM diazepam produced a slight increase in $t_{1/2}$. However, the glutamate synapse at the crayfish

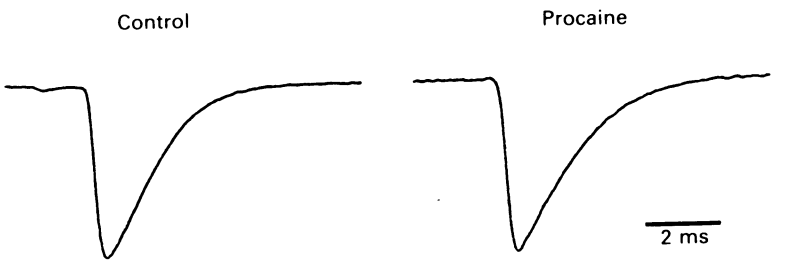


**Figure 3** Averaged excitatory junction currents recorded in the absence and presence of 0.5 mM procaine. In this experiment, $t_{1/2}$ increased by 11%, from 1.57 to 1.74 ms, while $A_I$ increased by 16%, from 1.82 to 2.10.

neuromuscular junction is relatively unaffected by these drugs, as compared with other preparations.

Procaine produced a slight change in the shape of the e.j.c., as evidenced by the finding that $A_I$ was increased slightly more than $t_{1/2}$. The effect was quite small, however, for a reasonably high concentration of procaine, and therefore the excitatory neuromuscular junction of the crayfish does not seem to be a suitable model system for studying the effects of procaine on glutamate-activated currents.

The volatile general anaesthetics ether and halothane caused a marked decrease in the duration of the decay phase of the e.j.c. Effects were observed at anaesthetic concentrations similar to those found clinically. Therefore, an increase in the rate of decay of glutamate-activated currents, and thus a decrease in the size of postsynaptic potentials produced by glutamate, may be significant during anaesthesia produced by these drugs. This finding may also be important when studying the responses of central neurones in anaesthetized animals.

Of course, one cannot necessarily draw conclusions concerning the pharmacology of glutamate responses in mammals based on experiments with crayfish. However, if the effects of the general anaesthetics are related to an increase in membrane fluidity (Gage & Hamill, 1976), then an acceleration of the rate of decay of excitatory postsynaptic responses may be a non-specific effect of the volatile anaesthetics. Depression of synaptic transmission by halothane and ether may occur at all types of synapses in the nervous system, and may not depend on the preparation being studied or the transmitter which produces the response.

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