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Frequency-dependent block of field potentials in the rat hippocampal slice caused by tricyclic antidepressants

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1 The effect of the tricyclic antidepressants imipramine and desipramine were studied on field potentials in the rat hippocampal slice. The electrically evoked stratum radiatum nerve volley, excitatory postsynaptic potential (e.p.s.p.) and pyramidal cell layer population spike (PS) were recorded in the CA1 region.

2 At concentrations of 10^{-6} M to 10^{-5} M, impramine did not affect the amplitude of the nerve volley, e.p.s.p. or PS at low frequencies of stimulation (0.01 Hz). At higher frequencies of stimulation (1–100 Hz), imipramine caused a frequency-dependent block of the nerve volley, e.p.s.p. and PS.

3 The time course of onset of the frequency-dependent block in the presence of imipramine was very slow. Maximum inhibition was reached after 3–4 h treatment with imipramine.

4 Desipramine (10^{-6}–10^{-5} M) also caused a frequency-dependent block of the hippocampal field potentials.

5 Only slight frequency-dependent block was observed in slices from rats injected *in vivo* with desipramine (10 mg kg^{-1}) for 14 days.

Introduction

Tricyclic antidepressant agents (TCAs) are well known to have a large number of actions on neurotransmitter systems in the rat brain. Acutely, TCAs have an inhibitory action on biogenic amine uptake and on the binding of dopamine, histamine and acetylcholine to their receptors (Sugrue, 1983; Willner, 1983; Janowski & Davis, 1980). Chronically, TCAs cause a number of adaptive changes in biogenic amine systems, including alterations in the number and responsiveness of β-adrenoceptors and 5-hydroxytryptamine (5-HT) receptors (Charney *et al.*, 1981; Sugrue, 1983; Olpe, 1983; Anwyl & Rowan, 1983; Rowan & Anwyl, 1984) and a reduction in the firing rate of noradrenaline containing neurones in the locus coeruleus (Svensson & Usdin, 1978).

TCAs are also known to have a local anaesthetic-type action on peripheral excitable tissue (Ritchie & Greengard, 1961; Seeman *et al.*, 1974; Schauf *et al.*, 1975; Wang *et al.*, 1981; Manzanares & Tamargo, 1983). This has led to the 'impulse blocking' hypothesis of antidepressant action (Randal, 1981), although it has generally been considered that concentrations of TCAs above the therapeutic range were necessary for neuronal block. There is now evidence that the therapeutic central nervous system (CNS) levels of TCAs in patients are about 10^{-6}–10^{-5} M (Biegon & Samuel, 1979a; Hrdina & Dubas, 1981; Glotzbach & Preskorn, 1982; Van Brunt, 1983; Kristinsson *et al.*, 1983). Recently, the inhibition of neuronal excitability by local anaesthetics has been shown to be of two types, a resting block and a frequency-dependent block (Strickhartz, 1973, Courtney, 1975). The frequency-dependent block can be produced by some of these agents at concentrations very much lower than those causing a resting block (Strickhartz, 1973, Courtney, 1975). The TCA imipramine has been shown to produce a frequency-dependent block of the cardiac muscle action potentials (Courtney, 1980). This may account for the antiarrhythmic effects of TCAs which are seen at therapeutic concentrations (Van Brunt, 1983).

In the present studies, we have investigated the action of TCAs on neuronal excitability in the CA1 area of the rat hippocampal slice. We demonstrate that acutely applied TCAs caused a frequency-dependent block of stratum radiatum compound action potentials and population excitatory postsynaptic potentials (e.p.s.ps), and also of pyramidal cell population

spikes (PS). This occurred at concentrations which did not affect the response to stimulation at frequencies of 0.01 Hz or less.

A preliminary account of some of these results has been published previously (Anwyl & Rowan, 1984).

Methods

Experiments were carried out on male albino Wistar rats (150–250 g). The methods for isolating and recording from hippocampal slices are well documented (Dingledine *et al.*, 1980). The rats were killed by decapitation. Transverse hippocampal slices (350 μm thick) were cut with a Campden Vibroslice at room temperature. Slices were completely submerged in a medium containing (mM): NaCl 120, KCl 2.5, $CaCl_2$ 2.0, $MgSO_4$ 1.0, $NaHCO_3$ 26, Na_2HPO_4 1.25 and glucose 10, which was flowing continuously at a rate of 10 ml min^{-1} and gassed with 95% O_2 plus 5% CO_2. The temperature of the medium was 35°C.

Glass microelectrodes (1–5 MΩ resistance) filled with 200 mM NaCl in agar were used for stimulating and recording. The stimulating electrode was placed in the stratum radiatum. Pulses were of 0.1 ms duration. A recording electrode was placed in the stratum radiatum to record the afferent nerve fibre volley and e.p.s.p., and in the stratum pyramidale to record the PS. In the stratum radiatum, when the stimulating and recording electrodes were placed close together, a single stimulus gave a stimulus artifact followed by an early negative wave, the nerve volley, and a second negative wave, the e.p.s.p. (Andersen *et al.*, 1978; Figure 1a). Experiments on the e.p.s.p. were carried out at e.p.s.p. amplitudes below the threshold for acion potentials so as to avoid alteration of the e.p.s.p. amplitude by the population spike.

The PS in the pyramidal layer is a negative wave superimposed on a positive wave, the e.p.s.p. (Figure 1b). The PS amplitude was measured as the mean of two amplitudes taken (a) from the peak of the initial positivity to the trough of the initial negativity, and (b) from the trough of the initial negativity to the peak of the second positivity.

The stratum radiatum fibres were initially stimulated at 0.01 Hz for several minutes to achieve a stable amplitude nerve volley, e.p.s.p. and PS. The frequency was then increased for 20 stimuli, and then returned to 0.01 Hz. The higher frequencies used were 1, 2, 5, 10, 20, 50 and 100 Hz. These were given in random order. An interval of 15 min was allowed between successive trains. Control recordings were made either 1–3 h or 5–7 h after commencing perfusion with the control medium. Imipramine was applied after the slices had been superfused with the control solution for 1 h.

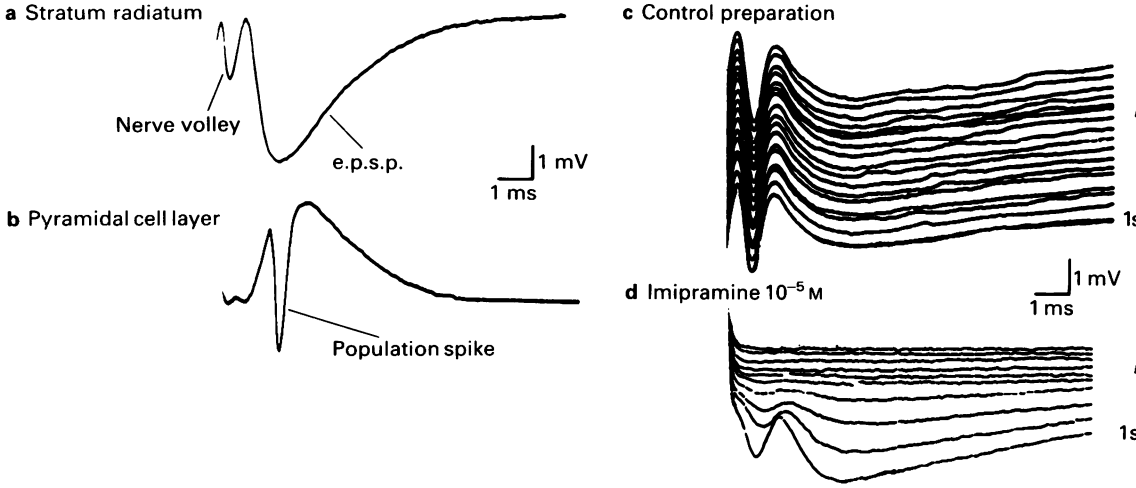

Figure 1 Recordings of electrically evoked field potentials in the CA1 region of the rat hippocampal slice. With the recording electrode in the stratum radiatum (a), the initial downward deflection is the afferent nerve volley. The subsequent slow downward deflection is the e.p.s.p. With the recording electrode in the pyramidal cell body layer (b), the e.p.s.p. was recorded as a slow upward deflection. When the stimulus intensity was above threshold a sharp downward deflection was superimposed on the e.p.s.p., the population spike. Recordings of the stratum radiatum nerve volleys and e.p.s.ps evoked by a train of stimuli at 100 Hz in the rat hippocampal slice (C) in a control preparation and (d) in a preparation which had been exposed to 10^{-5} M imipramine for 4 h. The bottom trace in (c) and (d) is the first in the train.

Table 1 Comparison of recordings of field potential responses to stimulation at 100 Hz in the rat hippocampal slice made at 1–3 h and 5–7 h after commencing perfusion

Field potential	*1–3* h	*5–7* h
Nerve volley	127 ± 4 (8)	121 ± 7 (5)
E.p.s.p.	60 ± 5 (8)	69 ± 5 (5)
Population spike	106 ± 7 (6)	107 ± 13 (5)

The values given are the amplitudes of the tenth potential in a train of 20 stimuli at 100 Hz. Amplitude is expressed as the percentage of the first potential in the train (± s.e.mean). The number of slices is given in parentheses.

In vivo *exposure to desipramine*

For the *in vivo* studies the rats were injected once daily with saline or desipramine (10 mg kg^{-1}) for 14 days. Slices were obtained 24 h after the last injection. A single slice was studied from each rat. Recordings were started 1 h after commencing perfusion.

Drugs

The following drugs were used: desipramine hydrochloride (Ciba-Geigy Pharmaceuticals, Horsham); imipramine hydrochloride (Sigma, London).

Statistics

Results are expressed as mean ± s.e. mean. The number of observations (n) is given in parentheses. The statistical significance of the differences between experimental groups was determined using Student's t test. A probability (P) value of 0.05 or less was taken to indicate statistical significance.

Results

Controls

Superfusion of the slices with the control medium for either 1 or 5 h before commencing stimulation did not significantly alter the recordings; therefore, the results from these two groups have been pooled (Table 1).

The nerve volley amplitude remained constant at stimulation frequencies of 1, 2, 5, and 10 Hz. However, at higher frequencies it increased by 5 ± 3% at 20 Hz, to a maximum of 30 ± 4% (n = 13) at 100 Hz (Figures 1c and 2a). No decline below the control amplitude was observed for the nerve volley at the frequencies tested.

The e.p.s.p. amplitude remained constant at stimulation frequencies of 1 and 2 Hz but always initially increased at higher frequencies. This initial increase reached a maximum of 40–50% by the 2nd–3rd stimulus at 50 Hz (n = 13). The e.p.s.p. amplitude always remained at or above the control level at frequencies up to and including 20 Hz. However, at 50 and 100 Hz the amplitude of the e.p.s.p. declined to 91 ± 4% (n = 13) and 57 ± 5% (n = 13) of the control value respectively by the 20th pulse (Figures 1c and 2a).

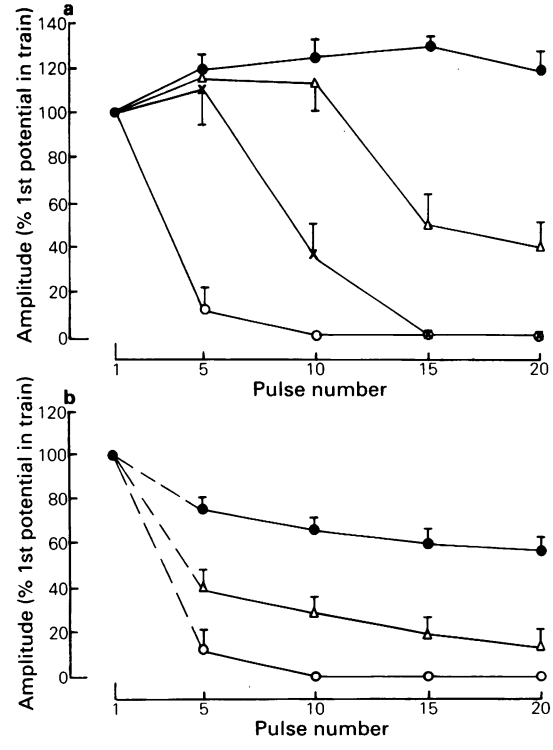

Figure 2 Concentration-dependence of the effect of imipramine on hippocampal slice field potentials evoked by trains of 20 stimuli at 100 Hz. (a) Stratum radiatum nerve volley. (b) Stratum radiatum e.p.s.p. Amplitudes are expressed as a percentage of the amplitude of the first potential in the train. The abscissa scale gives the pulse number in the train. Recordings were from untreated control preparations (●) n=13, or from preparations which had been pretreated with imipramine for 4–5 h at 2×10^{-6} M (△) n=4, 5×10^{-6} M (×) n=4, and 10^{-5} M (○) n=6. values are expressed as the mean and vertical lines show s.e.mean.

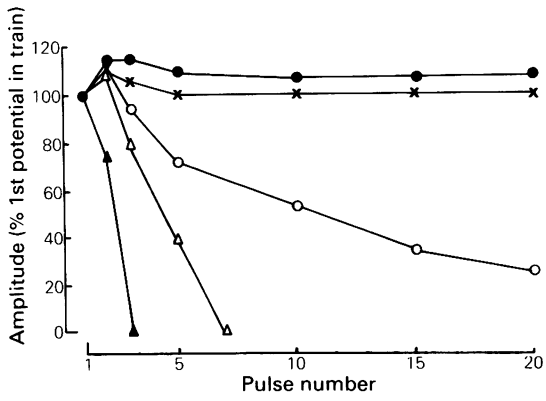

Figure 3 Time course of the effect of 10^{-5} M imipramine on pyramidal cell layer population spikes evoked by trains of 20 stimuli at 20 Hz. Population spike amplitude is expressed as a percentage of the amplitude of the first population spike in the train. The abscissa scale gives the pulse number in the train. Responses before (●) and 30 min (×), 90 min (○), 150 min (△) and 240 min (▲) after commencing perfusion with 10^{-5} M imipramine. Similar results were obtained in 3 other experiments.

The PS initially increased in amplitude at all frequencies from 1–100 Hz, with a maximum increase of 47 ± 4% (n = 11) present by the 3rd stimulus at 50 Hz. The PS declined after the 3rd stimulus at 50 and 100 Hz, but only at 100 Hz did the amplitude decrease below that of the first PS.

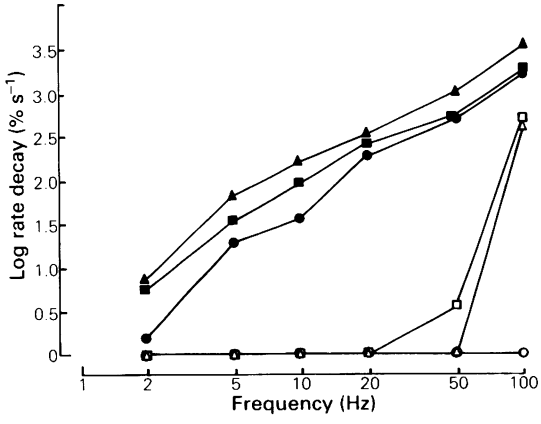

Figure 4 Frequency-dependence of the inhibitory effect of imipramine on field potentials in the hippocampal slice. The log rate of decay of the field potentials is plotted against frequency of stimulation. Control preparations: nerve volley (○), n=13; e.p.s.p. (□), n=13; population spike (△), n=11. Preparations treated with 10^{-5} M imipramine for 4–5 h: nerve volley (●), n=6; e.p.s.p. (■), n=6; population spike (▲), n=3. The s.e.mean were smaller than the symbols.

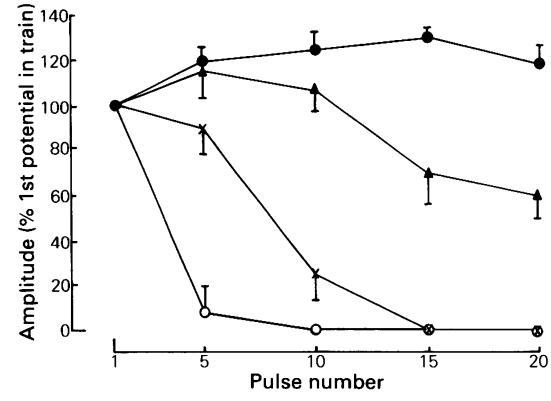

Figure 5 Concentration-dependence of the effect of desipramine on the stratum radiatum nerve volleys evoked by a train of stimuli at 100 Hz in the rat hippocampal slice. Nerve volley amplitude is expressed as a percentage of the amplitude of the first potential in the train. The abscissa scale gives the pulse number in the train. Recordings were from untreated control preparations (●), n=13, or from preparations which had been pretreated with desipramine for 4–5 h at 10^{-6} M (▲) n=3, 5×10^{-6} M (×) n=3, and 10^{-5} M (○) n=3. Vertical lines show s.e.mean.

Effects of bath applied imipramine

At the control stimulation frequency of 0.01 Hz, perfusion of imipramine at concentrations up to and including 10^{-5} M did not affect the amplitude of the stratum radiatum nerve volley, the radiatum e.p.s.p. or the PS. However, at frequencies of stimulation above 0.01 Hz, imipramine caused a decline in the amplitude of successive potentials in the train. The effect was greater at higher frequencies. This frequency-dependent block was concentration-dependent with a threshold of $1-2\times10^{-6}$ M. The time course for the development of the frequency-dependent block following perfusion of imipramine was very slow. Thus, effects were first observed 10–15 min after perfusion, but maximum inhibition was only reached 3–4 h after commencing perfusion (Figure 3).

The effects of 4–5 h perfusion of 2×10^{-6} M, 5×10^{-6} M and 1×10^{-5} M imipramine on a train of 20 responses evoked at a stimulation rate of 100 Hz are shown in Figures 1d and2. It can be seen that these concentrations of imipramine caused a decrease, or a further decrease, in the amplitude of the radiatum nerve volley and the e.p.s.p. when compared to controls. In the presence of 10^{-5} M imipramine, the amplitude of the nerve volley declined very rapidly following the first stimulus, and by the 10th stimulus had been reduced to zero. The decrease in amplitude of the e.p.s.p. closely matched that of the nerve volley at 100 Hz, while the PS declined at a much faster rate.

Table 2 The effect of long-term treatment of rats with desmethylimipramine (DMI) on field potentials evoked at different stimulation rates in the hippocampal slice

	10 hz		*100 Hz*	
Field potential	*Saline*	*DMI*	*Saline*	*DMI*
Nerve volley	105 ± 4 (5)	101 ± 2 (4)	125 ± 8 (5)	117 ± 2 (4)
E.p.s.p.	102 ± 3 (12)	97 ± 4 (4)	67 ± 4 (13)	44 ± 12* (5)
Population spike	99 ± 9 (9)	90 ± 2 (5)	105 ± 9 (7)	59 ± 13* (7)

Rats were injected daily with either saline or DMI (10 mg kg^{-1} i.p.) for 14 days. Hippocampal slices were taken 24 h after the last injection. The values given are the amplitudes of the tenth potential in the train at either 10 or 100 Hz. Amplitude is expressed as the percentage of the first potential in the train (± s.e.mean). The number of slices is given in parentheses. Only one slice from each rat was used.
* $P < 0.05$.

Thus in the presence of 10^{-5} M imipramine the PS amplitude decreased to zero by the 3rd stimulus.

The effect of different frequencies of stimulation on the rate of decrease of the amplitude of the nerve volley, the e.p.s.p. and the PS is shown in Figure 4. It can be seen that there was a very large difference at all frequencies between the control recordings and those made in the presence of 10^{-5} M imipramine. In the controls, decreases of amplitude were only seen at 50 and 100 Hz for the e.p.s.p. and at 100 Hz for the PS. However, in the presence of 10^{-5} M imipramine, all three potentials showed a decrease in amplitude at frequencies from 2 to 100 Hz. The rate of decrease of all three potentials was greater when the frequency of stimulation was raised. At 2–20 Hz the e.p.s.p. declined much more rapidly than the nerve volley. At 100 Hz the rate of decrease of the control e.p.s.p. and PS approached that seen in the presence of 10^{-5} M imipramine. However, at this frequency the amplitude of the potentials in the presence of imipramine reached zero during the train of stimuli (Figures 1d and 2b).

In three experiments, slices which had been superfused with 10^{-5} M imipramine for 3 h were washed with control solution for 1–2 h. No reversal of the frequency-dependent block was observed.

Effects of bath applied desipramine

Similar experiments were carried out in order to examine whether or not the tricyclic antidepressant desipramine also produced a frequency-dependent block of transmission. Bath application of 10^{-6}–10^{-5} M desipramine produced a frequency-dependent block of field potentials. Figure 5 illustrates the effects of different concentrations of desipramine on the nerve volley responses to stimulation at 100 Hz. It can be seen that desipramine was 1.5–2 times more potent than imipramine.

Effects of in vivo *injection of desipramine*

Slices from rats which had received a daily intraperitoneal injection of desipramine (10 mg kg^{-1} for 14 days) showed only a slight frequency-dependent block (Table 2). There was no apparent effect at stimulation frequencies below 100 Hz. At 100 Hz the inhibition was only statistically significant for the e.p.s.p. and population spike and not for the nerve volley.

Discussion

The present experiments demonstrated that the tricyclic antidepressant agents imipramine and desipramine produced a frequency-dependent block of the stratum radiatum nerve volley and e.p.s.p., and the pyramidal cell PS in the hippocampal slice preparation. This is consistent with previous reports of the effects of imipramine on cardiac and skeletal muscle (Courtney, 1980; Eldefrawi *et al.*, 1981). The frequency-dependent block of the nerve volley is most likely to be caused by a progressive blockade of the axonal Na^+ channels. It has previously been shown that certain local anaesthetics, anticonvulsants and antiarrhythmic drugs cause a frequency-dependent block of peripheral axonal Na^+ currents as well as causing a resting block of Na^+ currents (Courtney, 1975; Strickhartz, 1973; Courtney & Etter, 1983). The frequency-dependent block occurs at lower concentrations than the resting block, and is likely to be caused by preferential binding of the agent to Na^+ channels in the inactivated state. The axons in the stratum radiatum are known to conduct Na^+-mediated action potentials and are of very small diameter (0.1–0.2 μm) (Andersen *et al.*, 1978) and therefore may be particularly sensitive to inhibition by imipramine and

desipramine. It is known that the degree of resting inhibition of Na^+-mediated action potentials by anaesthetic-type agents is linearly related to the diameter of the axons, with the smallest being the most readily inhibited (Staiman & Seeman, 1977).

The e.p.s.p. amplitude was also reduced in a frequency-dependent manner by imipramine. At 50 and 100 Hz, the inhibition of the nerve volley and the e.p.s.p. were very similar, and it is therefore likely that the decrease in the e.p.s.p. was simply the result of the decrease in the presynaptic nerve volley. However, at 2–20 Hz, the e.p.s.p. in the presence of imipramine declined much more rapidly than the nerve volley, suggesting a possible action of imipramine on synaptic transmission. There is evidence that TCAs produce a time-dependent reduction of acetylcholine-mediated currents in frog skeletal muscle and *Aplysia* neurones by binding to the activated but non-conducting state of the receptor linked ionic channel (Aronstam, 1981, Eldefrawi *et al.*, 1981; Schofield *et al.*, 1981; Slater & Carpenter, 1982).

The frequency-dependent block of the PS was much greater than that of the nerve volley and e.p.s.p. This would be expected since the frequency-dependent block of action potentials in the pyramidal cell bodies would be additive with the e.p.s.p. inhibition.

The time course for the onset of the frequency-dependent block was very slow, with maximal block being reached 3–4 h after commencing perfusion with imipramine. This delay may be due to the time taken for imipramine to penetrate through the lipid membranes and subsequently to accumulate at some intracellular site of action. Evidence from studies on peripheral nerve fibres, squid axons and CNS neurones has established a likely intracellular site for the frequency-dependent blocking action of local anaesthetics. For example, QX314 and QX222, compounds that do not appreciably diffuse through cell membranes, caused a frequency-dependent inhibition of action potentials when injected intracellularly into hippocampal pyramidal neurones (Connors & Prince, 1982; Puil & Carlen, 1984). Moreover, it has been shown using autoradiographic techniques that TCAs accumulate intracellularly in central neurones (Biegon & Samuel, 1979a,b; Hrdina & Dubas, 1981). There is evidence that imipramine produces a resting block of conduction in peripheral nerves by acting intracellularly in its cationic form (Ritchie & Greengard, 1961).

In the present experiments there was only a small amount of frequency-dependent block of the e.p.s.p. and PS in hippocampal slices from rats treated chronically with desipramine. This is somewhat similar to the finding of Manzanares & Tamargo (1983) that 12–16 h after injection of rats with imipramine (7.5 mg kg^{-1} i.p. for 24 days) only relatively small inhibitory effects were detected in isolated cardiac muscle. The concentration of desipramine in the CNS has been found to be approximately 5×10^{-6} M 24 h after daily injection of desipramine, 10 mg kg^{-1} i.p. for 1–2 weeks (Vetulani *et al.*, 1976). The relatively small effects observed in the present experiments may be a result of washout of desipramine from the hippocampus during the dissection and perfusion period. However, in the acute *in vitro* experiments, the effects of imipramine were not easely reversed. *In vivo* recordings from rats chronically treated with desipramine have shown a reduction in the frequency of spontaneous action potentials in the cerebral cortex, cerebellum and locus coeruleus (Svensson & Usdin, 1978; Siggins & Schulz, 1979; Peterson *et al* 1983; Yeh & Woodward, 1983) but not in the hippocampus (Huang *et al.*, 1980).

TCAs are used in relatively high concentrations therapeutically, with plasma concentrations of 10^{-7}–10^{-6} M and brain levels reaching 10^{-6}–10^{-5} M (see Introduction for references). As these concentrations are of a similar order of magnitude to those found to produce frequency-dependent nerve block in the present experiments, it is possible that such a block may occur during therapeutic use of these drugs. It will be necessary to investigate more compounds in order to determine the extent to which this effect may play a role in the clinical situation. Evidence in support of such a role has recently been provided by Krijzen *et al.* (1983) who have shown that a wide variety of antidepressants (amitriptyline, chorimipramine, imipramine, iprindole and mianserin) exerted a powerful inhibitory effect on the hippocampal electroencephalogram of freely moving rats; this effect was selective to high frequencies (18 to 100 Hz).

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