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Increases in neuronal Ca^{2+} flux after withdrawal from chronic barbiturate treatment

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Abstract

Chronic barbital treatment significant increased the net K^+-stimulated uptake of $^{45}Ca^{2+}$ in cerebrocortical synaptosomal preparations, 24 h after withdrawal from chronic barbital administration. Basal uptake was not significantly changed. Hippocampal synaptosomal preparations showed a similar pattern, but the increase was not significant. The synaptosomal Ca^{2+} uptake was not affected by incubation with the dihydropyridine Ca^{2+} channel antagonist, nitrendipine, in controls or after chronic barbital treatment. Acute administration of a single dose of barbital did not alter the basal or stimulated uptake of $^{45}Ca^{2+}$ in cortical synaptosomes, when this was measured 36 h after the barbital administration. Hippocampal slices prepared 24 h after withdrawal from chronic barbital treatment showed a significant increase in K^+-stimulated uptake of $^{45}Ca^{2+}$, and the basal uptake was significantly decreased. Both changes were prevented by nitrendipine. An increase in the density of dihydropyridine-sensitive binding sites was found in the cerebral cortex. The results indicate that both dihydropyridine-sensitive and insensitive neuronal Ca^{2+} channels are altered by chronic barbiturate treatment. These changes may be involved in physical dependence on barbiturates.

Keywords: Barbiturate; Ca^{2+}; Withdrawal; Dihydropyridine

1. Introduction

Barbiturates have a range of acute pharmacological effects, but the basis of the tolerance and physical dependence is uncertain. They are well known to potentiate the effects of GABA (γ-aminobutyric acid), acting at their own receptor sites on the GABA/receptor ionophore complex (Olsen, 1981; Ticku and Maksay, 1984). Barbiturates block the activity at the kainate and AMPA (RS-α-amino-3-hydroxy-5-methyl-4-isoxole) subtypes of excitatory amino acid receptor, but have less effect at the NMDA (*N*-methyl-D-aspartate) subtype (Sawada and Yamamoto, 1985). Barbiturates also block Ca^{2+} uptake into neurones, when added acutely (Blaustein and Ector, 1975; Elrod and Leslie, 1980). The Ca^{2+} uptake induced by glutamate was found to be less affected by barbiturates than that produced by depolarisation (Harris and Stokes, 1982)

Voltage-sensitive Ca^{2+} channels are divided into subtypes, according to their activation and inactivation characteristics (Nowycky et al., 1985). Gross and Macdonald, 1988) found, using cultured neurones, that the effects of barbiturates were to block the 'L' subtype of channel and to increase the inactivation of the N subtype-of channel. The 'T' subtype of Ca^{2+} channel was not affected at the barbiturate concentrations studied. These corresponded to the general anaesthetic concentrations, and were higher than would be required to cause anticonvulsant actions. Ca^{2+} action potentials were also decreased by barbiturates (Morgan and Bryant, 1977; Heyer and Macdonald, 1982). Nishi and Oyama (1983) also found that barbiturates increased voltage-dependent inactivation of Ca^{2+} currents. Werz and Macdonald (1985), using mouse neurones in dissociated cell culture, concluded that barbiturates either enhanced Ca^{2+} channel inactivation or caused open channel blockade, at relevant concentrations relevant to their CNS (central nervous system) effects. An increase in rate of inactivation and in the proportion of Ca^{2+} channels inactivated was reported by Gundersen et al. (1988). This action was seen at concentrations that would be found in the CNS during the sedative and general anaesthetic actions of these drugs. The currents in the latter study possessed some of the characteristics of the 'N' subtype of Ca^{2+} channel, but were not identical with those described in cultured cells.

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The 'L' subtype of voltage-sensitive Ca^{2+} channels is selectively blocked by dihydropyridine compounds. We have found that these compounds have modulatory actions on ethanol dependence. Chronic ethanol treatment increased the number of high affinity binding sites for dihydropyridines in the central nervous system (Dolin et al., 1987). When these compounds were given concurrently with the ethanol, they prevented the development of tolerance (Wu et al., 1987; Little and Dolin, 1987; Dolin and Little, 1989) and the ethanol withdrawal syndrome (Whittington et al., 1991), and the increase in binding site number was also prevented. These effects were suggested to be due to an adaptive response to the dihydropyridine, preventing the upregulation of dihydropyridine-sensitive binding sites, as the central concentrations during measurement of tolerance and withdrawal were too low to have any acute actions (Dolin and Little, 1989; Whittington et al., 1991).

Concurrent chronic administration of a dihydropyridine Ca^{2+} channel antagonist, with a barbiturate significantly decreased the signs of the barbiturate withdrawal syndrome, but did not alter barbiturate tolerance (Rabbani et al., 1994). Little is known about the effects of long-term barbiturate administration on Ca^{2+} channels. The present study measured the effects of chronic barbiturate treatment on $^{45}Ca^{2+}$ uptake into synaptosomes and into tissue slices, the effects of dihydropyridine Ca^{2+} channel antagonists on this uptake, and the effects of chronic barbiturate treatment on central dihydropyridine binding.

2. Materials and methods

2.1. Chronic drug treatment

Barbital was used for the chronic barbiturate treatment, because this compound does not induce microsomal enzymes. Male mice of the TO strain were used, between 25 and 30 g. They were given barbital in powdered food for 7 days: 3 mg barbital per g food for 2 days, 4 mg g food^{-1} for 2 days and 5 mg g food^{-1} for 3 days. The mean intake of barbital was 400 mg kg^{-1} day^{-1} at the beginning of the treatment, rising to 700 mg kg^{-1} day^{-1} at the end. Controls received a matched amount of powdered food only. All mice were weighed regularly during the treatments and no significant differences in weights were found. In all studies the amount of food, and hence barbital, taken in by the mice was measured daily. Our previous work has shown that this dose schedule produced tolerance to barbiturates and a clear withdrawal syndrome on cessation (Rabbani et al., 1994).

2.2. Ca^{2+} uptake into synaptosomes

Synaptosomes were prepared from cortices or hippocampi of TO mice using the method of Gray and Whittaker (1962) with minor modifications. Nerve terminal-enriched material in the 0.8 M fraction from a sucrose gradient, was equilibrated by addition of 25 ml of basal Krebs/Tris solution containing: 135 mM NaCl; 5 mM KCl; 1.2 mM $MgCl_2$; 1.25 mM $CaCl_2$; 10 mM glucose; and 20 mM Tris. The pH was adjusted to 7.7 at 37°C. The stimulatory Krebs/Tris solution contained 70 mM KCl and 70 mM NaCl, with the other salts remaining the same. The choice of 70 mM KCl was made on the basis of prior studies that showed this concentration produced a just submaximal increase in Ca^{2+} uptake, compared with 5 mM KCl.

The diluted synaptosome suspension was centrifuged at $14{,}000 \times g$ for 20 min and pellet was resuspended in basal Krebs/Tris solution. Synaptosomal suspension (200 μl) was preincubated with 50 μl of dihydropyridine or vehicle on ice for 20 min. The dihydropyridines were dissolved in 0.5% DMSO (dimethyl sulphoxide) (final DMSO concentration 0.05%), prior to addition to the incubation medium at 10 μM. Where Cd^{2+} was used it was included in the preincubation medium at 200 μM. The preincubated tubes were then placed in a water bath for 10 min at 37°C. The $^{45}Ca^{2+}$ uptake was initiated, following pre-incubation, by addition of 250 μl of basal or stimulatory buffer containing $^{45}Ca^{2+}$ (2 μCi ml^{-1}). The reaction was terminated after 1 s by dilution with 2 ml of cold lanthanum 'stop' buffer (this medium was prepared by mixing the same components as the Krebs/Tris buffer with 1 mM $LaCl_3$ substituted for the $CaCl_2$). After termination of the reaction, the diluted suspension was filtered through Whatman GF/B filters in a Brandel M30-R Cell Harvester followed by two 4 ml washes with ice-cold lanthanum 'stop' buffer, carried out within 10 s. Liquid scintillation counting was then carried out on the filters in plastic vials containing 4 ml of scintillant (Unisolve E). The net K^+-stimulated uptake was calculated by subtraction of basal uptake from the uptake in the stimulatory buffer.

2.3. Ca^{2+} uptake into hippocampal slices

Hippocampal slices, 300 μm thick, were prepared in standard Krebs solution (composition below) 24 h after withdrawal from the chronic barbital treatment. The slices were preincubated for 10 min at 37°C in Krebs solution gassed with 95% O_2/5% CO_2. Where nitrendipine or Cd^{2+} were used, they were included in the preincubation medium, at 10 μM and 200 μM, respectively. The slices were then incubated for 10 min at 37°C in $^{45}Ca^{2+}$ and Krebs solution with a K^+ concentration of either 5 mM or 70 mM, with or without nitrendipine or Cd^{2+} as above. Five slices were used for each *n* value, in 1-ml Krebs solution. At the end of this period, 4 ml ice-cold lanthanum chloride 'stop' buffer (10 mM $LaCl_3$, 1.2 mM $MgSO_4$, 5 mM KCl, 135 mM NaCl and 20 mM HEPES) was added to each set of five slices. The tissues were then immediately filtered under suction and washed three times

with the 'stop' buffer, then each set of five was transferred to vials containing 500 μl 1 M NaOH. They were incubated at 60°C for 70 min, then 3 × 50 μl samples removed for the protein assay. Four-milliliter scintillation fluid was added to the remaining solution and the $^{45}Ca^{2+}$ counted. The protein concentrations were estimated using Commassie blue reagent (Bradford, 1976), and the net K^+-stimulated uptake was calculated by subtraction of basal uptake from the uptake in the stimulatory buffer.

A prior measurement of the time course of Ca^{2+} uptake by the slices demonstrated basal and stimulated Ca^{2+} uptake that reached a maximum at 10 min, after which the rate of stimulated uptake declined. The time interval of 10 min was therefore chosen for this study.

The composition of the Krebs solution was as follows, (mM): NaCl 124, KCl 3.25, KH_2PO_4 1.25, $NaHCO_3$ 20.0, $MgSO_4$ 2.0, $CaCl_2$ 2.0 and D-glucose 10.0. When the K^+ concentration was raised to 70 mM, the concentration of sodium was decreased accordingly.

2.4. Dihydropyridine binding

Dihydropyridine radioligand binding assays (Glossman and Ferry, 1985) were performed on cerebral cortices taken from mice 24 h after withdrawal from chronic barbital treatment. The mice were killed by cervical dislocation and the cerebral cortices removed and frozen. Frozen–thawed tissues were homogenised in 50 mM Tris–HCl (pH 7.4, 25°C) in a glass hand-held homogeniser. The homogenate was centrifuged at 30,000 × *g* for 15 min and the resultant pellet washed twice and finally suspended in 50 mM Tris–HCl. The crude membrane suspension (0.1–0.3 mg ml^{-1}) was incubated in the presence of [^{3}H]nitrendipine (84 μCi mol^{-1}) 0.125–12 nM, at a final volume of 0.5 ml in borosilicate glass tubes. Nonspecific binding was defined in the presence of an excess of unlabelled nimodipine, 1 μM. Incubation was performed in the dark, at 25°C for 45 min. The reaction was terminated by rapid filtration through Whatman GF/B filters in a Brandel M30-R Cell Harvester, followed by two 4-ml washes with cold assay buffer, carried out within 10 s. Filters were placed in plastic vials with 4 ml of scintillant (Unisolve E) and counted on a Packard 1900CA counter. Binding data from each tissue was analysed by fitting to a rectangular hyperbola binding isotherm equation ('GraphPAD Inplot' program) and hence determining the affinity constant (K_d) and the number of binding sites (B_{max}).

2.5. Analysis of results

Comparisons in both the receptor binding studies and the Ca^{2+} uptake measurements were made by Student's *t*-test. The uptake into hippocampal tissues was also compared by two-way analysis of variance (ANOVA). Because there were different numbers in each treatment group, a nonparametric analysis of variance was used (Meddis, 1984), as the parametric two-way analysis cannot be applied in these circumstances. The *n* values were 22 and 17, respectively for the control and barbital treatments in the binding study, each measurement being made on tissues from a different animal. Five to seven measurements, each on tissues from different animals, were used in the Ca^{2+} uptake studies. When cortical synaptosomes were prepared, the tissues from two mice were used for each *n* values. In the experiments on hippocampal synaptosomes, the hippocampi from 15 mice were used for each estimation.

3. Results

3.1. Ca^{2+} uptake by synaptosomes

The effects of the chronic barbital treatment on $^{45}Ca^{2+}$ uptake by cortical synaptosomes, measured 24 h after cessation of the treatment, are illustrated in Fig. 1. Chronic barbital treatment did not significantly change the basal uptake, although the mean values were slightly increased (not illustrated). The barbital treatment significantly increased the net $^{45}Ca^{2+}$ uptake stimulated by K^+, by 27% ($P < 0.05$, $n = 6$).

Addition of the Ca^{2+} channel antagonist, nitrendipine, to the incubation medium did not significantly alter the basal or stimulated uptake, in either control or barbital treated tissues (Fig. 1), and the increase seen after the barbital treatment was still apparent in the presence of

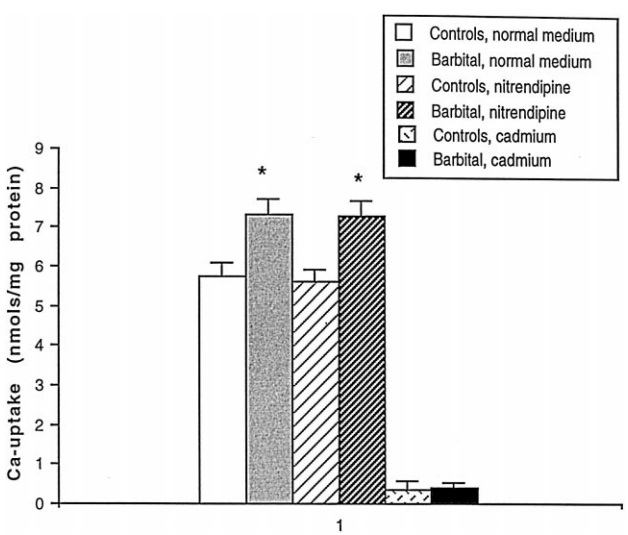

Fig. 1. The effects of chronic barbital treatment on basal (a) and net K^+-stimulated (b) $^{45}Ca^{2+}$ uptake into cerebrocortical synaptosomes. (a) shows the basal Ca^{2+} uptake, 24 h after cessation of the 7-day barbital diet. The differences seen between the control and barbital-treated tissue were not significant. Chronic barbital treatment significantly increased ($P < 0.05$) the K^+-stimulated Ca^{2+} uptake, at 24 h withdrawal (b). The uptake was unaltered by the addition of 10 μM nitrendipine to the incubation medium, in control tissues or after chronic barbital administration. Values are means ± S.E.M.

these compounds. The $^{45}Ca^{2+}$ uptake was inhibited by Cd^{2+}, a nonspecific Ca^{2+} channel antagonist, which blocked 30% of basal and 94% of net stimulated uptake in control tissues, indicating that the stimulated flux occurred through Ca^{2+} channels.

Synaptosomes prepared from hippocampi removed 24 h after withdrawal from chronic barbital treatment showed a similar pattern of $^{45}Ca^{2+}$ uptake as the cortical tissue (Fig. 2), but in this case the increase in net uptake stimulated by K^+ seen after the barbital treatment did not quite reach significance ($P > 0.05$). No significant effect of nitrendipine was seen, in controls or after barbital treatment.

To determine whether or not the increase in the $^{45}Ca^{2+}$ uptake was due to the chronic administration of barbital, the effects of a single barbital dose were studied. Cortical synaptosomes were prepared 36 h after an i.p. injection of 150 mg kg^{-1} barbital. The basal Ca^{2+} uptake was unchanged ($P > 0.1$) after the barbital administration (Fig. 3). There were no significant differences ($P > 0.1$) between the K^+-stimulated $^{45}Ca^{2+}$ uptake in tissues from vehicle-injected or from barbital treated mice (Fig. 4). Nitrendipine, added to the incubation medium, did not alter the $^{45}Ca^{2+}$ uptake by control or barbital-treated tissues.

3.2. Ca^{2+} uptake by hippocampal slices

The time of 10 min for incubation with $^{45}Ca^{2+}$ was chosen on the basis of preliminary experiments, that indicated that the Ca^{2+} uptake reached a maximum value at this time. Prior studies showed that the 70 mM KCl concentration produced a stimulation of Ca^{2+} uptake that was just submaximal and that the addition of Cd^{2+} blocked the net stimulated uptake by 95% and the basal uptake by 8%.

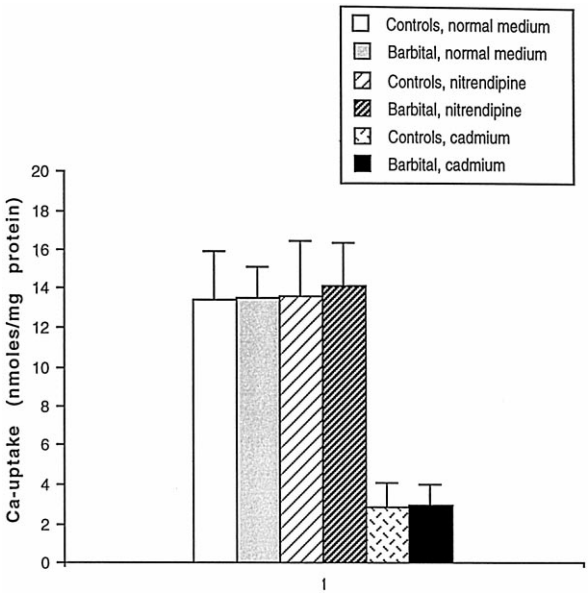

Fig. 3. The effects of a single dose of barbital on Ca^{2+} uptake. Thirty-six hours after barbital, 150 mg kg^{-1}, the basal Ca^{2+} uptake (Fig. 4a) and K^+-stimulated Ca^{2+} uptake (b) were unaltered. The uptake was not changed by the addition of 10 μM nitrendipine to the incubation medium, in control tissues or after barbital administration. Values are means ± S.E.M.

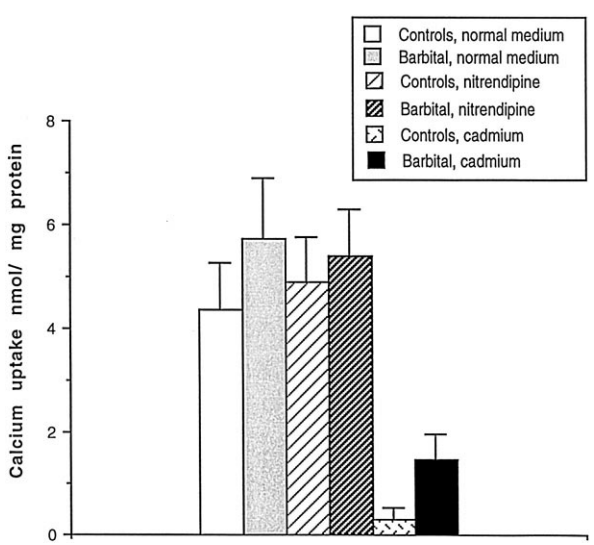

Fig. 2. The effects of chronic barbital treatment on basal (a) and K^+-stimulated (Fig. 3b) synaptosomal $^{45}Ca^{2+}$ uptake into hippocampal tissues. The differences between the control and barbital-treated tissues in the basal or stimulated Ca^{2+} uptake, in the absence of dihydropyridines 24 h after cessation of the 7-day barbital diet, were not significant ($P > 0.05$). The uptake was unaltered by the addition of 10 μM nitrendipine to the incubation medium, in control tissues or after chronic barbital administration. Values are means ± S.E.M.

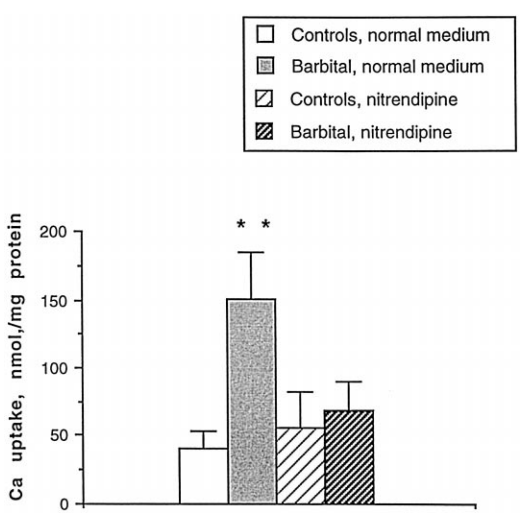

Fig. 4. Uptake of $^{45}Ca^{2+}$ into hippocampal slices prepared 24 h after withdrawal from chronic barbital treatment. (a) shows that basal uptake into barbital treated tissues was significantly decreased, but nitrendipine added to the incubation medium had no effect on this uptake in either case. Uptake stimulated by K^+, however, (b) was significantly increased and this increase was completely prevented by 10 μM nitrendipine. $^{*}P < 0.05$, for comparison between control and barbital treated tissues in the absence of nitrendipine. $^{**}P < 0.01$, for comparison between control and barbital treated tissues in the absence of nitrendipine, and $P < 0.05$ for comparison between the presence and absence of nitrendipine for barbital treated tissues.

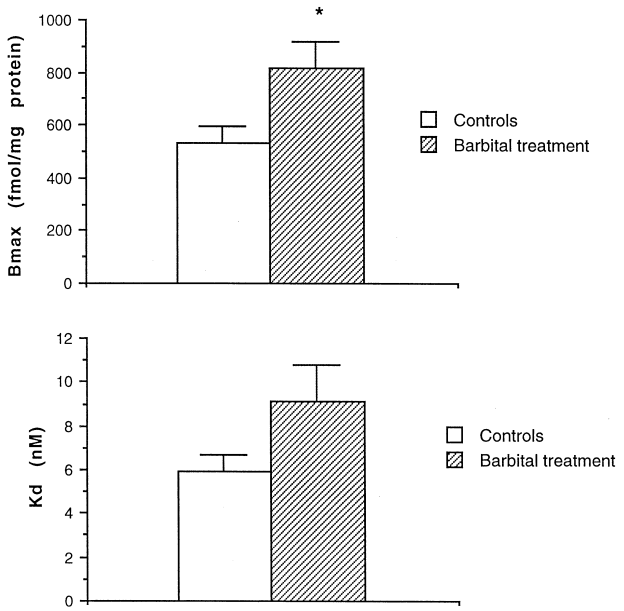

Fig. 5. Measurements of dihydropyridine binding in cerebral cortical tissues, prepared 24 h after cessation of chronic barbital treatment. (a) shows the B_{max} values and (b) the K_d values. Values are means ± S.E.M. *: $P < 0.01$ compared with control values.

The basal uptake of $^{45}Ca^{2+}$ into hippocampal slices was decreased by the chronic barbital treatment (Fig. 4a); the decrease just reached statistical significance ($P < 0.05$). This pattern was not seen in the presence of nitrendipine; there were no significant differences between the uptake in the presence and absence of nitrendipine, in control or barbital treated tissues. There was a large increase in the Ca^{2+} uptake stimulated by K^+ to 376% of control values (Fig. 4b) that was statistically significant ($P = 0.01$). When 10 μM nitrendipine was included in the incubation medium, this increase was completely abolished (Fig. 4b). The difference between the uptake in barbital treated tissues in the presence and absence of nitrendipine was significant ($P < 0.05$).

3.3. Dihydropyridine binding

Twenty-four hours after cessation of chronic barbital treatment there was a 54% increase in B_{max} values for dihydropyridine binding in cerebral cortex, compared with concurrently treated controls (Fig. 5a). This difference was significant ($P < 0.01$; $n = 22$ and 17, respectively). The K_d values, were not significantly altered by the barbital treatment although the mean values were higher after the barbital treatment (Fig. 5b).

4. Discussion

The results showed that chronic barbital treatment increased stimulated neuronal Ca^{2+} uptake in cerebrocortical tissue. Depolarisation-induced synaptosomal uptake was increased after the chronic barbital treatment, and there was no effect of the Ca^{2+} channel antagonist, nitrendipine. The latter result is in agreement with previous data, which demonstrated that dihydropyridine sensitive Ca^{2+} channels are located on the soma and dendrites of neurones (Sanna et al., 1986; Westenbroek et al., 1990). Most studies have not found any effects of dihydropyridines on synaptosomal Ca^{2+} uptake, but Turner and Goldin (1985) found that the rapid phase of uptake (1–10 s) was blocked by nitrendipine. Woodward and Leslie (1986) demonstrated stimulation of uptake by Bay K 8644 (±methyl-1.4 dihydro-2,6-dimethyl-3-nitro-4-(trifluoromethylphenyl)-pyridine-5-carboxylate) in striatal synaptosomes, that was seen only at a K^+ concentration of 15 mM. The increase in the Ca^{2+} uptake after the chronic barbiturate treatment therefore indicates that there were changes in Ca^{2+} flux in the terminal areas, that did not involve dihydropyridine-sensitive channels. The functional Ca^{2+} channels in nerve terminals are thought to be mostly of the 'N' subtype (Hirning et al., 1988), and it is possible that this subtype, or other non-dihydropyridine-sensitive channels are involved in the increased reuptake into synaptosomes after the barbital treatment.

The concentration of nitrendipine used in the present study was based on earlier work on the effects of this compound on CNS tissues. The concentrations of dihydropyridine Ca^{2+} channel antagonists that are required to affect central neurones are higher than those which block Ca^{2+} channels in peripheral tissues. The concentration used in the present studies has been shown to have neuronal effects, decreasing the hyperexcitability seen in hippocampal neurones during withdrawal from chronic ethanol treatment (Whittington and Little, 1991a), and was in the range demonstrated to be found in the brain after intraperitoneal doses that produced behavioural effects (Whittington et al., 1991). The protective effect of dihydropyridine Ca^{2+} channel antagonists on hippocampal neurones were demonstrated to be stereospecific and due to effects on voltage-sensitive Ca^{2+} channels (Whittington and Little, 1991b, 1993). Other actions of dihydropyridine Ca^{2+} channel antagonists have been reported on central neurones, at concentrations higher than those effective in peripheral tissues (Middlemiss and Spedding, 1985; Raeburn and Gonzales, 1988; Grover and Teyler, 1990; Simmons et al., 1995). Preliminary work in our laboratory has shown that higher concentrations of nitrendipine than that used in the present study will decrease Ca^{2+} uptake into synaptosomes from control animals, but such concentrations were so high (100 μM and above) that they would have had nonspecific actions.

The lack of changes after a single dose of barbital indicated that the increase observed after the chronic barbiturate treatment was an adaptive response occurring during the chronic administration. It is likely that this was in response to the acute effect of the barbiturate in decreasing

Ca^{2+} conductance (Gross and Macdonald, 1988); most authors have found that tolerance occurs to the acute blockade of uptake by barbiturates (Elrod and Leslie, 1980; Harris and Stokes, 1982). Previous studies have not found changes in synaptosomal $^{45}Ca^{2+}$ uptake after chronic barbital treatment, although Leslie et al. (1980) reported an increase in mean values 13 days after phenobarbital administration that did not quite reach significance. The rats in that study were not withdrawn from the barbiturate before synaptosome preparation, and whole brain tissue was used. It is likely that the duration of withdrawal would affect the measurements. In the study of Jones and Beaney (1980) the animals were not withdrawn from the chronic barbiturate treatment and no changes in Ca^{2+} uptake were found. However, in the work of these authors, *n* values of only 3 were used. Ondrusek et al. (1979) prepared whole brain synaptosomes 20 h after withdrawal from 7 days phenobarbital treatment, but no differences in $^{45}Ca^{2+}$ uptake were found. Another important difference from our results was the duration of incubation; we used 1-s incubation times, while earlier work used longer times, for example, Ondrusek et al. (1979) measured over 1 min. Nachshen and Blaustein (1980) showed that the fast phase of uptake, thought to represent uptake through voltage-sensitive channels, lasts only 1 s, so the very short incubation time used in the present study would have measured only this phase.

The basal uptake of Ca^{2+} represents uptake that is not voltage-dependent, and is probably mostly due to exchange mechanisms (Nachshen and Blaustein, 1980), rather than uptake through voltage-sensitive Ca^{2+} channels. Consistent with this were the results showing that the basal uptake was little affected by Cd^{2+}. The basal uptake was not significantly changed after the barbital treatment in cortical or hippocampal synaptosomes, although the mean values were consistently higher than controls.

The hippocampal synaptosomes did not show a significant increase in stimulated Ca^{2+} uptake, indicating that there may be different patterns of changes in different brain regions, although the mean value was higher after the barbital treatment. The effects of K^+ may differ between tissues; a full K^+ concentration effect curve was not established in hippocampal synaptosomes because of the large number of animals required to provide each *n* value (see Section 2). The reason for using hippocampal tissue was to determine whether the differences between the lack of effect of nitrendipine on cortical synaptosomes and its effectiveness in hippocampal slices was a tissue or a preparation difference. The results, however, were equivocal, suggesting that both factors may affect the results.

The results from the measurement of Ca^{2+} uptake into hippocampal slices showed that the K^+-stimulated uptake was considerably increased after the chronic barbital treatment and that this increase was sensitive to dihydropyridine blockade. K^+-stimulated uptake into control tissues was unaffected by nitrendipine, suggesting that the increase in uptake may have been due to the activity dihydropyridine-sensitive channels that do not normally contribute to the uptake. The basal uptake after chronic barbital treatment was decreased, but this pattern of results was not seen after addition of nitrendipine; the reason for this latter effect is not clear, but the differences were small, compared with those seen in stimulated uptake.

As described above, dihydropyridine-sensitive Ca^{2+} channels are known to be located on soma and dendrites of neurones (Sanna et al., 1986; Westenbroek et al., 1990). In order to determine whether there were changes in these in cerebrocortical tissue, that were not detected in the Ca^{2+} uptake studies on synaptosomes, dihydropyridine binding was measured in the cerebral cortex. The number of dihydropyridine-sensitive binding sites was increased, but the affinity was not significantly changed, although the mean value was raised. Our earlier studies demonstrated increased density of dihydropyridine binding in cerebral cortex after chronic ethanol treatment, but little change in affinity (Dolin et al., 1987). Barbiturates have been shown to have direct effects on dihydropyridine binding (Harris et al., 1985; Hirota and Lambert, 1996), but it is unlikely that in the present study residual barbital would have had direct effects on the receptor, as the brain levels of barbital 24 h after withdrawal would have been very low (P.V. Taberner, personal communication). The lack of action of nitrendipine on the K^+-stimulated uptake into synaptosomes and the increase in dihydropyridine binding are compatible with the known locations of the Ca^{2+} channel subtypes.

Cross-dependence between ethanol and barbiturates has been reported on many occasions. However, although the behavioural signs of withdrawal from chronic barbiturate and chronic ethanol treatments are similar, differences have been reported. Marietta et al. (1989) showed phenobarbital withdrawal caused a pattern of cerebral glucose utilisation different from that previously reported for ethanol withdrawal, and Okamoto et al. (1983) found that diazepam was more effective in protecting against the barbiturate than the ethanol withdrawal syndrome. As mentioned in the introduction, chronic ethanol intake increased the number of dihydropyridine binding sites, with no change in affinity. The present results support the concept that the withdrawal syndromes caused by ethanol and by barbiturates may share some common mechanisms, although our behavioural results (Rabbani et al., 1994) suggested that there were differences in the ways in which Ca^{2+} channels were involved.

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