after 60 min of exposure, since the effects of highly lipophilic drugs such as propranolol and alprenolol became more prominent and deviated from the regression line. On the basis of 'modulated receptor hypothesis' in nerves[5-7], the resting block in the present experiments may be interpreted in the following 2 ways; a) only a fraction of the drug molecules is diffused away, during a long pause in stimulation, from the binding sites; this may have some bearing on the gating mechanism of the Na channels; and/or b) they associate rapidly with the sites at the moment of an initiation of action potential despite a complete loss of drug molecules at the receptor site during a pause, which may be the case with lidocaine[5] ('apparent' tonic block). At present, it can not be decided which mechanism plays a greater role. It is well known that hydrophilic β-blockers, such as sotalol, practolol and INPEA[13], and atenolol[14] have only weak or no quinidine-like activity. Sada et al.[15] demonstrated that the order of potency for the depressant effects of several β-blockers on $\dot{V}_{max}$, evaluated in the myocardium at 1 Hz, did not run completely in parallel with the lipophilic properties of the drugs. These results suggest that the time-independent effects of these drugs are determined mainly by the lipophilic property of the drug molecules in cardiac tissues, as is the case with nerve fibres[6,7], whereas these effects are more-or-less modified by the rate-dependent effects at the rate of 1 Hz or those rates at which the latter effects were operative. Finally, we must point out the possibility that these drugs may not affect the sodium channels directly but affect some other currents, by which the sodium channel is secondarily affected[16].

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Direct excitant action of convulsant barbiturates[1]

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Summary. 5-(2-cyclohexylideneethyl)-5-ethylbarbituric acid (CHEB) and other convulsant barbiturates produced depolarization of isolated dorsal root fibres and other unmyelinated nerves from the rat. CHEB-induced depolarizations, unlike kainate-induced depolarizations were abolished by omission of Ca^{2+} from, or addition of ruthenium red (5 µM) to, the bathing medium.

The action of depressant barbiturate drugs is produced largely through potentiation[5-7] and/or mimicry[8,9] of the effects of the inhibitory neurotransmitter γ-aminobutyric acid (GABA)[10]. However, some barbiturate derivatives have a convulsant action[11] which is unlikely to be mediated by the same mechanisms that give rise to depressant action. The convulsant 5-(2-cyclohexylideneethyl)-5-ethyl barbituric acid (CHEB) has been reported to directly depolarize dorsal root ganglion cells, in the cat, thereby evoking spontaneous discharge in the peripheral ends of cut dorsal roots[12].

In the present experiments a series of barbiturate and non-barbiturate convulsants have been examined for their ability to depolarize isolated dorsal root fibres from the rat. Only barbiturates with convulsant activity in vivo produced depolarization of dorsal root fibres and other unmyelinated nerves. Furthermore, depolarizations of dorsal root fibres induced by CHEB, unlike kainate-induced depolarizations, were abolished by omission of Ca^{2+} from, or addition of ruthenium red (5 µM) to, the bathing medium. It is suggested that the convulsant barbiturates represent a hitherto undescribed class of excitants which depolarize cell membranes through a calcium-dependent mechanism.

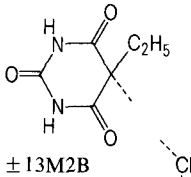
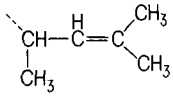
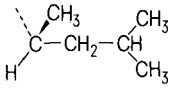
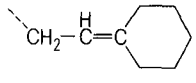
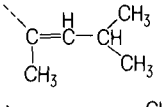
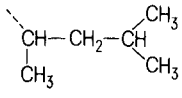
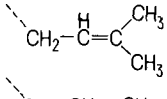
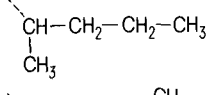
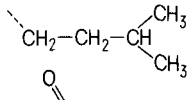
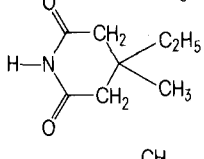
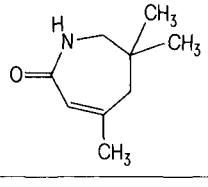
The method of recording electrical polarity of spinal roots and other nerves, the composition of the bathing medium and the method of application of drugs have been described previously[13]. The action of convulsant barbiturates on motoneurones, as recorded from ventral roots, of hemisected spinal cords of immature (2–9-day-old) rats was similar to that of kainic acid[14] (figure, a). However, an action of these convulsants different from that of excitant amino acids is indicated because CHEB and other convulsant barbiturates were found to depolarize ventral roots isolated from immature rats and vagus nerve, sympathetic nerve and dorsal roots of mature rats; all of these being preparations which are not depolarized by excitant amino acids[15,16]. A phrenic nerve and ventral root from a mature rat were unaffected by CHEB (500 µM). The threshold concentration for the depolarization of sympathetic nerves (superior cervical pre- and post-ganglionic trunks) and dorsal roots of mature rats was relatively high (200 µM CHEB) while that for the vagus nerve was lower (25 µM CHEB). The preparation most sensitive to the depolarizing action of these compounds (threshold 2.5 µM CHEB) was the dorsal root of the immature rat (figure, b), consequently this preparation was

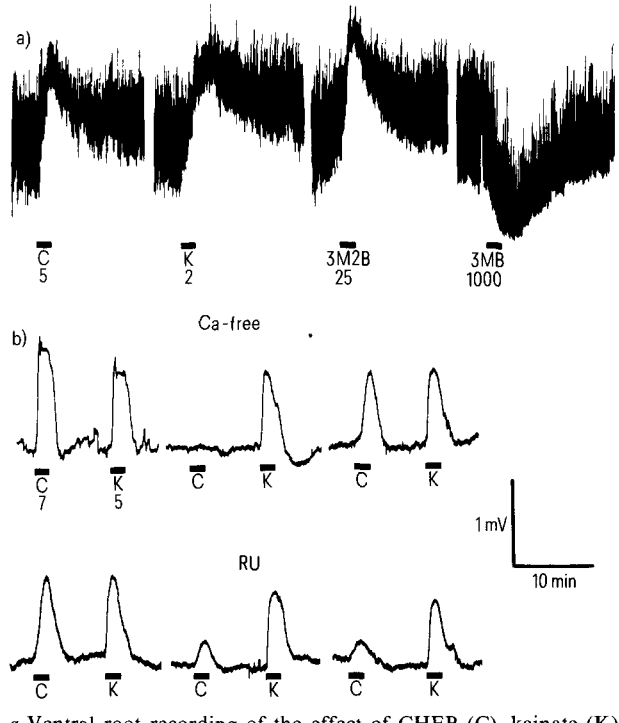
used for the subsequent studies including determination of the depolarizing potencies listed in the table. The action of these compounds on vagus and sympathetic nerve and the absence of effect on phrenic nerve suggests that sensitivity to these agents may be a general property of unmyelinated nerve fibres. Depressant barbiturates will directly depolarize sympathetic ganglia and dorsal root fibres by a picrotoxin or bicuculline-sensitive mechanism[3,17,18]. The time course of this effect is slower than that reported here for convulsant barbiturates and the effect is not easily recorded at the chart speed (4 mm/min) used during the present study. The depressant barbiturates pentobarbitone (±1 MB) and amylobarbitone (3 MB) (table) were tested at 500 µM on 4 preparations and on 2 of these preparations both produced small depolarizations which were abolished by picrotoxin (50 µM). Depolarizations elicited by the convulsants 3M2B and CHEB were unaffected by bicuculline hydrochloride (100 µM) or picrotoxin (100 µM).

The results summarized in the table indicate that the depolarizing action reported here correlates with the in vivo convulsant action of barbiturates. Depressant barbiturates and the non-barbiturate convulsants tested (bemegride[19] and 4,6,6-trimethyl-Δ3-caprolactam[20], see table) were devoid of depolarizing action. Thus it is unlikely that convulsant barbiturates share a common mode of action with the convulsant glutarimides or caprolactams despite their apparent structural resemblance.

Convulsant barbiturates contract various smooth muscle preparations[21] and CHEB-induced contractions of aortic strips are dependent on exogenous Ca^{2+}[22]. In the present experiments omission of Ca^{2+} from the bathing medium abolished the depolarization of dorsal roots induced by CHEB but not by kainate (figure, b). The omission of Ca^{2+} produced an immediate hyperpolarization (not shown in figure, b) which was restored to the control level by a corresponding depolarization on readmission of 2.5 mM

Comparison of chemical structure equimolar depolarizing potency and equimolar convulsant potency. Depolarizing potencies are means ± SE of mean of determinations from 4 preparations. The relative potencies were calculated from the concentration of each compound necessary to match responses evoked by CHEB at 2–4 times threshold concentration (see figure, b). The comparable value obtained for kainic acid was 2.23 ± 0.21. NE = no effect.
Equimolar convulsant potencies were calculated from the median convulsant dose in mice (CD_{50} mg/kg i.p.) using tonic extensor seizure as the endpoint[7,22].

Compound	Depolarizing potency	Convulsant potency
		
±13M2B 	4.00 ± 0.34	2.88 ± 0.20
+13MB 	2.23 ± 0.21	1.46 ± 0.08
CHEB 	1.00	1.00
13M1TB 	0.30 ± 0.04	0.51 ± 0.04
±13MB 	0.27 ± 0.03	0.51 ± 0.05
3M2B 	0.21 ± 0.03	0.58 ± 0.05
±1MB 	NE	Depressant
3MB 	NE	Depressant
Bemegride 	NE	1.30
4,6,6-trimethyl-Δ3-caprolactam 	NE	3.80 ± 0.95



a Ventral root recording of the effect of CHEB (C), kainate (K), 3M2B and amylobarbitone (3MB) on motoneurones of a hemisected spinal cord from an immature rat. *b* Recording from an isolated dorsal root from an immature rat. Ca^{2+}-free medium was introduced 4 min before the start of the top centre trace. Recovery from Ca^{2+}-free medium is shown 9 min following return to the control medium. 1 µM ruthenium red (RU) was introduced 5 min before the start of the lower centre trace and the lower right hand trace was obtained 20 min following return to the control medium. The ruthenium red used was from a commercial preparation of 20% purity. – Depolarization is indicated by upward deflection on the records and agonists were applied for the period indicated below the records, concentration shown in µM.

Ca^{2+}. This indicates that membrane Ca^{2+} permeability has a significant effect on the resting membrane potential of the fibres in these preparations. Ruthenium red inhibits synaptosomal membrane Ca^{2+} flux[23]. Treatment of 2 dorsal root preparations with ruthenium red (1 µM) depressed responses to CHEB irreversibly and in 2 preparations treated with 5 µM ruthenium red CHEB-induced responses were abolished with no recovery up to 60 min after washout of the dye. Responses induced by kainate were unaffected by ruthenium red treatment (figure, b). Treatment of dorsal root preparations with 10 mM, but not 1 mM, Mg^{2+} had a similar selective blocking action against responses induced by CHEB.

In conclusion, the results show that the convulsant properties of the barbiturate analogues listed in the table are clearly related to their potencies as direct excitants and that this excitation probably results from an influx of Ca^{2+}. Convulsant barbiturates have been shown to inhibit a Ca^{2+}-dependent ATPase, believed to represent a Ca^{2+} pump, in synaptosomal membranes[24].

Inhibition of such a membrane Ca^{2+} pump might explain the Ca^{2+} dependence of the depolarizing action of these compounds. Since neurotransmitter release is dependent on Ca^{2+} influx[25], transmitter release from nerve terminals might be stimulated in the presence of CHEB, or other barbiturate convulsants. The convulsant action probably results from this effect in combination with excitation resulting from direct depolarization of neurones[12].

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Methyl cellosolve-induced sensitization of mice to bacterial endotoxin

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Summary. Treatment of mice with a sublethal dose of methyl cellosolve renders the animals 64-fold more sensitive to the toxic effects of bacterial endotoxin.

In the course of studies on drug-endotoxin interactions in mice, methyl cellosolve was used to solubilize certain hydrophobic drugs. It soon became clear that the solubilizing agent itself increased the lethality of endotoxin and this report will describe the magnitude and conditions of the sensitization due to methyl cellosolve.

Materials and methods. Male, NMRI strain mice weighing between 18 and 22 g were injected i.v. with various doses of *S. marcescens* endotoxin suspended in 0.25 ml deionized water. Immediately following the endotoxin, mice received i.v. injections (0.25 ml) 1 M methyl cellosolve or 1 M propylene glycol. Evaluation of the LD_{50} was based on the 7-day mortality and was calculated by probit analysis of the dose[3].

Methyl cellosolve (ethylene glycol monomethyl ether) was purchased from Fisher Scientific Company, Fair Lawn, NJ, and propylene glycol was obtained from Mayco Industries Inc., Philadelphia, Pa. The *S. marcescens* endotoxin (lot No. 508948) was a Boivin preparation obtained from Difco Laboratories, Detroit, MI.

Observations and discussion. Mice receiving endotoxin alone exhibited an LD_{50} of 17 mg/kg (table). Treatment of mice with 1 M propylene glycol, in addition to endotoxin, did not change the sensitivity of the mice to endotoxin as shown by the 16 mg/kg LD_{50} in these animals. However, mice treated with endotoxin, followed immediately by 1 M methyl cellosolve (i.v.), showed a reduction in the LD_{50} to 0.25 mg/kg. This represents a 64-fold sensitization in the methyl cellosolve-treated animals. The injection of 0.25 ml of 1 M methyl cellosolve represents a dose of 950 mg/kg on a weight basis, and this amount of methyl cellosolve caused no deaths when injected into 20 mice.

Further characterization of the drug-endotoxin interaction was undertaken by modifying the route of administration and the time interval between endotoxin and methyl cellosolve treatment. The data in the table show that i.p.