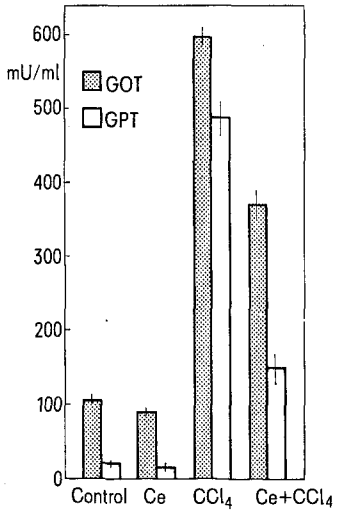
CCl_4 alone, the centrolobular necrosis was more severe than in liver of rats pretreated with cerium chloride and intoxicated with CCl_4.

The toxicity of CCl_4 is assumed to be due to reactive metabolites, such as $\cdot CCl_3$ free radical, which are produced in the DMES[3]. Since cerium chloride depresses the DMES[1], it may be implied that protection by cerium chloride in CCl_4-intoxication may be due to a diminished biotransformation of the haloalkane in the liver. A protective effect has been shown by us[11] and CARLSON[12] with lead nitrate and methylmercury respectively. Also in these cases, protection is due to inhibition of the DMES induced by the metals, and consequently to reduction of CCl_4 metabolism into toxic products.



Serum transaminases glutamic oxaloacetic (GOT) and glutamic pyruvic (GPT) under the following experimental conditions: Cerium chloride was given i.v. at the dose of 4.0 mg/kg b.wt., 72 h before intoxication; CCl_4 was given p.o. at the dose of 2.5 ml/kg b.wt. The rats were sacrificed 24 h after intoxication. Vertical bars represent the SEM from at least 6 rats.

Table II. Protection by cerium chloride on the liver polysomal damage induced by CCl_4

Treatment	Polysomes[a] / Total ribosomes
a) Control	0.59 ± 0.02
b) Cerium chloride	0.59 ± 0.08
c) CCl_4	0.39 ± 0.05
d) Cerium chloride + CCl_4	0.53 ± 0.05

[a] Calculated by the areas of the polysomal patterns. Mean $\pm$ SE. 5 animals were used for each group. CCl_4 was given p.o. at the dose of 2.5 ml/kg b.wt., 1 h before sacrifice. Statistical significance by t-test was: a–c; c–d: $p < 0.001$.

[11] P. PANI, A. SANNA, F. P. CORONGIU and L. CONGIU, Drug Metab. Dispos. *3*, 148 (1975).
[12] G. P. CARLSON, Toxicology *4*, 83 (1975).

Depression of Neurones in the Rat Cerebral Cortex by Leptazol

T. W. STONE

Physiology Department, University of Aberdeen, Marischal College, Aberdeen AB9 1AS (Scotland), 18 August 1975.

Summary. The convulsant drug leptazol was applied by microiontophoresis to 116 neurones in the cerebral cortex of rats. The firing of 101 cells was reduced. Only 6 cells were excited.

Pentamethylenetetrazol (leptazol) is a convulsant drug whose mode of action is unknown. Several other convulsants act by interfering with the actions or release of inhibitory neurotransmitters in the central nervous system. Strychnine blocks the postsynaptic actions of glycine[1] and both picrotoxin and bicuculline antagonise γ-aminobutyric acid[2]. Leptazol does not antagonize central inhibitory transmitters in this way[3,4] and it has been suggested that leptazol could have a direct excitatory action on some cells[5–8]. The present experiments were undertaken to examine this possibility.

Materials and methods. Male hooded Listar rats weighing 250–300 g were anaesthetized with urethane, 1.25 g/kg i.p. Experiments were performed in the somatosensory cerebral cortex on cells which were either randomly encountered at various depths or identified as pyramidal tract cells by antidromic stimulation of the medullary pyramid[9].

Details of the preparation of animals, and of the microiontophoretic techniques used have been given previously[9]. Five-barrelled micropipettes with overall

[1] D. R. CURTIS, A. W. DUGGAN and G. A. R. JOHNSTON, Expl Brain Res. *12*, 547 (1971).
[2] D. R. CURTIS, A. W. DUGGAN, D. FELIX and G. A. R. JOHNSTON, Brain Res. *12*, 69 (1971).
[3] D. R. CURTIS, Pharm. Revs. *15*, 333 (1963).
[4] R. D. HILL, M. A. SIMMONDS and D. W. STRAUGHAN, Br. J. Pharmac. *49*, 37 (1973).
[5] E. B. KIRSTEN and E. P. SCHOENER, Neuropharmacology *11*, 591 (1972).
[6] J. LEWIN and D. W. ESPLIN, J. Pharmac. exp. Ther. *132*, 245 (1961).
[7] K. NEGISHI, M. C. BRAVO and M. VERZEANO, Electroenceph. clin. Neurophysiol. Suppl. *24*, 90 (1963).
[8] M. VERZEANO, R. NAQUET and E. E. KING, J. Neurophysiol. *18*, 502 (1955).
[9] T. W. STONE, J. Physiol., Lond. *225*, 485 (1972).

tip diameters of 4–8 μm have been used, containing solutions of: sodium L-glutamate 200 m*M*, pH 7.5; acetylcholine chloride 200 m*M*, pH 4.0;(–) noradrenaline bitartrate 200 m*M*, pH 4.0; 5-hydroxytryptamine creatinine sulphate 50 m*M*, pH 4.5; pentamethylenetetrazol 200 m*M*, pH 5.5.

Current balancing was practized routinely to eliminate artifactual changes of firing rate caused by the passage of iontophoretic current[8]. Recording of cell activity was achieved through a single micropipette electrode (tip 1 μm) secured alongside the multibarrel assembly[10]. Direct recordings of cell firing frequency were obtained on a Servoscribe pen recorder, recording the output from an Ekco Instantaneous Ratemeter. All the cells studied were spontaneously active.

Results. Leptazol did not excite many cortical units. When ejected with outward iontophoretic currents of 20–100 nA for periods of between 5 sec and 10 min, the only change seen on 101 (87%) of the 116 cells studied was a depression of firing rate. This depression was apparent soon after beginning the iontophoretic ejection, the response latencies being in the range of 2–14 sec. A typical response record is illustrated in Figure 1B.

Only 6 units (5%) were encountered which were excited by leptazol. An example of such a response, a slowly developing low amplitude excitation, is shown in Figure 1A. It is interesting to note that of the 35 pyramidal tract cells tested, 32 (92%) were depressed by leptazol and none were excited. This raises the possibility that the excitant effects of leptazol may have been indirect effects, due to the drug's diffusing to and inhibiting a neurone which was not being recorded but which was inhibiting the cell being recorded.

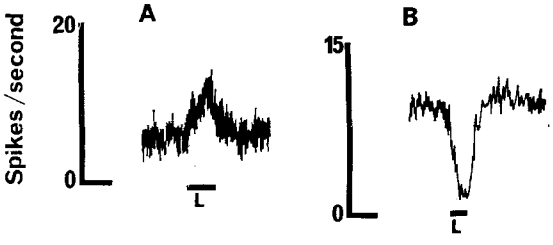


Fig. 1. Records of the firing rate of units which are excited (A) and depressed (B) by the iontophoresis of leptazol (L) ejected with a current of 60 nA. Time: 1 min.

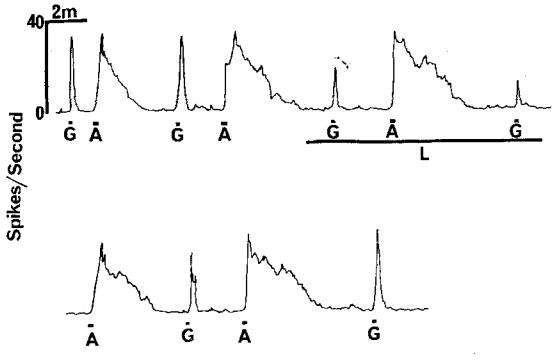


Fig. 2. Records of the firing rate of a pyramidal tract cell, antidromic latency 2.10 msec, which was excited by acetylcholine 60 nA (A), and glutamate 60 nA (G). The ejection of leptazol 60 nA (L) reduces the peak firing rate produced by glutamate by approximately 50%, whilst having little effect on the acetylcholine response. The 2 records are continuous. Time: 2 min.

The neuronal depression by leptazol could also be seen as a reduction of glutamate-induced firing on 45 cells which were subjected to regular iontophoretic pulses of glutamate (Figure 2). Excitation by acetylcholine was also frequently reduced by leptazol (41 cells), though this reduction was much less than for glutamate, and on 5 cells there was little apparent change in the acetylcholine response (Figure 2).

The reduction of glutamate firing by leptazol could not be attributed to either a local anaesthetic action of the convulsant or to a rapid overdepolarization, since photographic records showed no change of spike height during the action of the agent.

Depressant responses to the iontophoresis of acetylcholine (11 cells), noradrenaline (14 cells) or 5-hydroxytryptamine (8 cells) were unaffected by leptazol.

The finding of depressant responses to leptazol is not likely to be an artifact of our experimental design as KRNJEVIĆ et al.[11] in their study of the pharmacology of cortical inhibition, applied leptazol to some cells and commented that glutamate responses were 'actually depressed in some ... cases'.

HILL et al.[4] also found that 4 of 12 cells studied were depressed by leptazol. If the present experiments have any relevance to leptazol's convulsant properties it may be that in the central nervous system as a whole leptazol causes depression of firing of some cells, including inhibitory interneurones and as a result there is an overall reduction of inhibition, leading to an increase of ongoing activity.

A previous report of the effects of systemic injections of leptazol[5] stated that 90% of the cells studied (in the vestibular nucleus) were excited and 10% were depressed by the injection. The former was attributed to a direct excitatory action of leptazol, and the latter to an indirect action (excitation of inhibitory neurones). We would suggest from the evidence of the present experiments that the depressions may be the more 'direct' response, and the excitations the result of an inhibition of inhibitory interneurones.

The possibility that excitatory responses to leptazol may be indirect effects has been mentioned above. However, several groups using the readily accessible neurones of *Aplysia* and *Helix* have reported that leptazol can induce paroxysmal depolarizations with the production of high frequency bursts of action potentials[12–15]. It is possible therefore that in the cerebral cortex both excitatory and inhibitory effects of leptazol are genuine direct effects, perhaps on different types of cells. No excitatory responses were seen on identified pyramidal tract cells.

Nevertheless it should be emphasized that almost all workers using leptazol have observed hyperpolarizing or inhibitory responses as well as depolarizations[13, 14, 16].

[10] T. W. STONE, J. Physiol., Lond. *233*, 211 (1973).
[11] K. KRNJEVIĆ, M. RANDIĆ and D. W. STRAUGHAN, J. Physiol., Lond. *184*, 78 (1966).
[12] R. J. DAVID, W. A. WILSON and A. V. ESCUETA, Brain Res. *67*, 549 (1974).
[13] M. R. KLEE, D. S. FABER and W. D. HEISS, Science *179*, 1133 (1973).
[14] E. J. SPECKMAN and M. CASPERS, Epilepsia *14*, 397 (1973).
[15] W. A. WILSON and A. V. ESCUETA, Brain Res. *72*, 168 (1974).
[16] J. W. PRICHARD, Brain Res. *27*, 414 (1971).