Short communications

Antagonism of GABA by picrotoxin in the feline cerebral cortex

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Picrotoxin and γ-aminobutyric acid (GABA) were applied by microiontophoresis to an unselected population of neurones in the feline cerebral cortex. Picrotoxin was shown to antagonize the depressant effects of GABA and in addition appeared free of some of the actions of bicuculline which tend to mask GABA antagonism.

Picrotoxin has been shown to be a potent and specific antagonist of the effects of γ-aminobutyric acid (GABA) at invertebrate synapses (Robbins & van der Kloot, 1958; Takeuchi & Takeuchi, 1969; Walker, Crossman, Woodruff & Kerkut, 1971). There is also evidence that picrotoxin is a relatively selective antagonist of the depression produced by microiontophoresis of GABA onto brain stem and spinal cord neurones in cats (Galindo, 1969; ten Bruggencate & Engberg, 1971; Engberg & Thaller, 1970; ten Bruggencate & Sonnhof, 1971). However, no convincing evidence of the antagonism of GABA depression of cortical neurones by picrotoxin was found (Krnjević, Randić & Straughan, 1966); a view confirmed by Krnjević (1970) in a recent review. The present study now provides some of the hitherto missing evidence for the effectiveness of microiontophoretically applied picrotoxin as a GABA antagonist in cat cortex.

Methods.—Cats, of either sex, were anaesthetized with halothane in N_2O/O_2. Mid suprasylvian or pericruciate gyri of the cerebral cortex were exposed by removal of the overlying skull and dura and the position of the brain was stabilized with a celluloid pressor.

Seven barrelled glass micropipettes were used, of tip diameter 6 ± 2 μ. Extracellular action potentials were recorded via one barrel, containing 3 M NaCl solution and a second NaCl barrel was used to balance the currents applied to the remaining, drug containing, barrels.

The drug solutions used were sodium l-glutamate (0·2 M pH 8), GABA (0·2 M, pH 3·5), glycine (0·2 M, pH 3·5), picrotoxin (5 mM in 165 mM NaCl with no adjustment of pH) and bicuculline (5 mM in 165 mM NaCl at pH 3·5). All ionized drugs were retained by a 25 nA backing current of appropriate polarity.

Results and Discussion.—An unselected population of neurones was studied at depths ranging from 200 to 2,000 μm below the cortical surface, and all units encountered were depressed by GABA applied from the pipette. Glycine could not be used routinely as a control agonist in these experiments since responses to the large currents required to cause 100% inhibition were followed by greatly delayed recoveries. All cells studied were firing in response to a low continuous current of glutamate, and depressant responses to GABA were studied before, during and after application of picrotoxin.

Figure 1 (A) shows a rate meter record from one cortical neurone, and the reversible effect of picrotoxin in antagonizing depression produced by a low current of GABA can clearly be seen.

In Fig. 1 (B) are shown cumulative dose-response curves (see Straughan, Neal, Simmonds, Collins & Hill, 1971) constructed from single applications of GABA to the same cell, as depicted in Figure 1 (A). A higher current of GABA was used so that 100% inhibition of firing was produced even during the picrotoxin application. Again it can clearly be seen that picrotoxin reversibly antagonizes the depression produced by GABA.

A total of 16 neurones was studied in this way and the mean shift to the right of the dose-response line for GABA (expressed as a ratio of the times to reach 50% inhibition of firing) was 3·1 for picrotoxin applied with a current of 50 nA. In 9 of the 16 cells, ratios greater than 2·0 were obtained. This should be compared with a mean ratio of 1·5 for 30 neurones to which bicuculline was applied with a current of 50 to 75 nA, in exactly similar experiments (all potentiations of GABA by bicuculline excluded) (Straughan *et al.*, 1971; Hill, Simmonds &

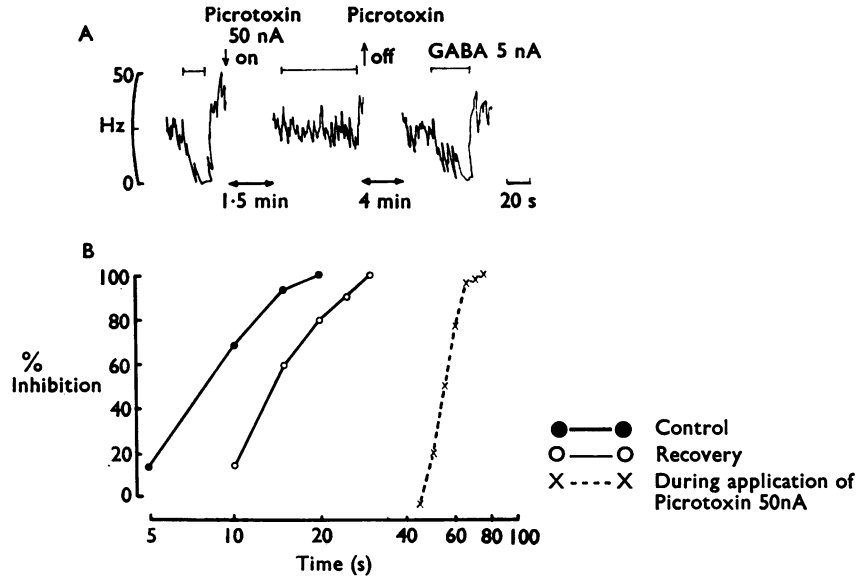

FIG. 1. (A) Analogue rate meter record of the firing of a cell driven by the continuous application of 10 nA glutamate, at a depth of 1·34 mm in the suprasylvian cortex of a cat. GABA 5 nA was applied as indicated by the bars above the trace. Picrotoxin 50 nA was applied continuously between the vertical arrows (see text). (B) Dose-response curves constructed from spike counter data obtained from the same cell. The GABA current was increased to 10 nA in order to achieve 100% depression during picrotoxin application. The shift ratio produced by picrotoxin in this case was 6·1 (see text).

Straughan, 1971). The ratios for bicuculline were greater than 2·0 in only 7 out of the 30 neurones.

In addition, picrotoxin, unlike bicuculline, has shown no tendency to potentiate the effects of GABA (Straughan *et al.*, 1971; Godfraind, Krnjevic & Pumain, 1970). A further advantage of picrotoxin is that it does not alter the efflux of ^{3}H-GABA from rat cortical slices, whereas bicuculline has now been found to increase ^{3}H-GABA efflux (Alexander & Neal, unpublished results).

No conclusions on the specificity of picrotoxin for GABA receptors on cortical neurones can be drawn from our experiments, as we were unable to obtain reliable depressions with glycine microiontophoresed on to these neurones (see Curtis, Duggan, Felix, Johnston & McLennan, 1971). However, in the spinal cord and Deiter's nucleus, convincing evidence of picrotoxin being selective for GABA has been obtained (Engberg & Thaller, 1970; ten Bruggencate & Engberg, 1971) as glycine will readily depress neurones in these areas.

Picrotoxin appears to be free from some of those properties of bicuculline which may mask antagonism of GABA, and it appears that picrotoxin applied iontophoretically remains at least as useful an agent as bicuculline for antagonizing the central effects of GABA.

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'Since the submission of this paper, a report has appeared (JOHNSTON, G. A. R. & MITCHELL, J. F. (1971), *J. Neurochem.* **18**, 2441–2446) confirming that bicuculline can increase the efflux of ^{3}H-GABA from rat cortical slices.'