ANTAGONISM OF INHIBITORY AMINO ACID ACTION BY TUBOCURARINE

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A comparison has been made of the antagonism by microelectrophoretically administered (+)-tubocurarine, bicuculline methochloride and strychnine of the inhibition of spinal interneurones and Renshaw cells in the cat by glycine and γ-aminobutyric acid. The results indicate that (+)-tubocurarine would be of little use in assessing which of these amino acids was the transmitter at central inhibitory synapses.

Tentative identification of the transmitter released at the terminals of a particular set of nerve fibres is frequently based upon the effects of antagonists of known transmitters and of their interaction with compounds which mimic transmitter action when artificially administered. In microelectrophoretic investigations of this type in the central nervous system, the practical value of an antagonist depends on both its potency and the degree of selectivity it exhibits towards the effects of substances other than the transmitter.

Glycine and γ-aminobutyric acid (GABA) have been proposed as important inhibitory transmitters within the mammalian central nervous system (see Curtis & Johnston, 1974). There is now a considerable body of evidence, largely derived from microelectrophoretic experiments, that strychnine and bicuculline are adequately selective glycine and GABA antagonists, respectively, to provide reliable information regarding the probable nature of an inhibitory transmitter. In the case of bicuculline corroborative evidence has generally been obtained by the use of picrotoxinin, although the selectivity of the latter as an amino acid antagonist requires further investigation (Curtis & Johnston, 1974).

Doubt has been expressed, however, in a recent publication (Hill, Simmonds & Straughan, 1973b) regarding the usefulness of bicuculline as a GABA antagonist, and the claim has been made that (+)-tubocurarine is not only more potent than bicuculline (and picrotoxin) as an antagonist of the inhibitory action of GABA on the firing of cat cortical neurones, but additionally is more useful 'in terms of practical microelectrophoresis'. This proposal disregarded the 'marked' excitant effect of tubocurarine on cortical neurones and the little, if any, selectivity demonstrated between the inhibitory actions of glycine and GABA (also Hill, Simmonds & Straughan, 1972; 1973a). This apparent lack of specificity contrasts with previously reported observations of the selective antagonism by bicuculline of the inhibitory action of GABA-like amino acids upon neurones in the cat cerebral cortex, other supraspinal regions (Curtis, Duggan, Felix, Johnston & McLennan, 1971b) and the spinal cord (Curtis, Duggan, Felix & Johnston, 1971a). Consequently, tubocurarine has been examined as an amino acid antagonist in the cat spinal cord, and in particular its activity as a GABA antagonist has been compared with that of a more soluble bicuculline analogue, bicuculline methochloride (BMC) (Johnston, Beart, Curtis, Game, McCulloch & Maclachlan, 1972).

Methods The methods used to study the chemical sensitivity of spinal interneurones and Renshaw cells of cats anaesthetized with pentobarbitone sodium have been described previously (Curtis *et al.*, 1971a). Extracellular action potentials of single neurones were recorded by the centre barrel (4 M NaCl) of seven barrel micropipettes and active ions were administered electrophoretically from aqueous solutions within the other barrels: DL-homocysteate (0.2 M, pH 7.5, NaOH); acetylcholine (bromide, 0.5 M); γ-aminobutyric acid (GABA, 0.5 M, pH 3, HCl); glycine (0.5 M, pH 3, HCl); bicuculline methochloride (BMC, 10 mM in 165 mM NaCl); strychnine (hydrochloride, 2 mM in 165 mM NaCl) and (+)-tubocurarine (hydrochloride, approximately 70 mM in 165 mM NaCl). As in earlier studies the neurones were either firing spontaneously or were excited by the continuous ejection of DL-homocysteate or acetylcholine, such currents being adjusted when necessary to maintain rates of firing during the administration of antagonists similar to those during control periods of observation. Currents ejecting glycine and GABA were chosen to produce just maximal or submaximal depression of firing, usually within 10 s, and equilibrium firing rates were used to determine the effectiveness of the amino acids and antagonists.

Results The most commonly observed effect of tubocurarine when administered with cationic currents of 20-300 nA was a reduction of the

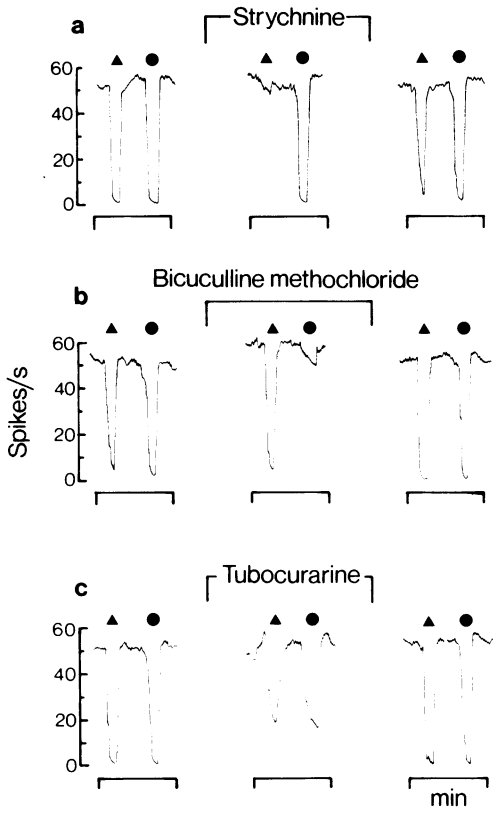

Fig. 1 Maximal effects of (a) strychnine, (b) bicuculline methochloride and (c) (+)-tubocurarine on the inhibition of firing of a spinal interneurone by glycine (▲, 8 s) and GABA (●, 10 s). Firing was maintained at a constant level with DL-homocysteate. From left to right: control responses; during administration of the indicated alkaloid; recovery.

(a) Glycine, 15 nA; GABA, 20 nA; strychnine (2 mM in 165 mM NaCl), 40 nA for 2 minutes. Recovery at 5 min was incomplete.

(b) Record 6.25 min after (a). Glycine, 15 nA; GABA, 12.5 nA; bicuculline methochloride (10 mM in 165 mM NaCl), 40 nA for 4 minutes. Recovery 2 minutes.

(c) Two minutes after (b). Glycine, 15 nA; GABA, 12.5 nA; (+)-tubocurarine (70 mM in 165 mM NaCl), 100 nA for 3 min after 40 nA for 2 min, 60 nA for 3 min, 80 nA for 2 minutes. Recovery 2 minutes.

inhibitory action of both GABA and glycine. This *non-specific* antagonism was observed with 48% (2 Renshaw cells, 10 interneurones) of cells from a total of 25 tested in nine cats. Tubocurarine had no effect on the inhibitory action of either GABA or glycine upon 32% (3 Renshaw cells, 5 interneurones) of the cells, whilst it antagonized only glycine on 12% (1 Renshaw cell, 2 interneurones) and only GABA on 8% (2 interneurones). In comparison, BMC (25-75 nA) specifically antagonized the inhibitory action of GABA on 14 of the 15 cells (4 Renshaw cells, 10 interneurones) tested with both tubocurarine and BMC. Allowing a factor of 7 for the differences in concentration of the tubocurarine and BMC solutions, the latter was invariably more potent than tubocurarine as a GABA antagonist. Strychnine (15-50 nA) also consistently and specifically antagonized the inhibitory action of glycine on all (2 Renshaw cells, 9 interneurones) cells tested with both tubocurarine and strychnine. These effects of BMC and strychnine were observed irrespective of the type of effect that tubocurarine had on the actions of GABA and glycine. Almost all cells were excited by tubocurarine, and this effect appeared to be unrelated to the type of amino acid antagonism observed. Figure 1 compares the effects of BMC, strychnine and tubocurarine on the inhibitory actions of GABA and glycine upon a spinal interneurone. Whereas BMC and strychnine antagonized inhibition of firing by GABA and glycine respectively, tubocurarine reduced the inhibitory actions of both amino acids.

Discussion These results indicate that relative to strychnine and bicuculline methochloride, the potency and selectivity of (+)-tubocurarine as an antagonist of inhibitory amino acids are both so low that this alkaloid could not be considered suitable for determining whether a glycine- or a GABA-like amino acid was the transmitter at central inhibitory synapses. No support can be offered for the proposition that (+)-tubocurarine is a *useful* GABA antagonist (Hill *et al.*, 1973b): indeed it might be questioned whether the methods used by Hill *et al.* (see Hill & Simmonds, 1973), which depend on modification by an antagonist of the time-response curves of electrophoretically administered agents, can provide insight into the 'utility' of antagonists. This type of observation may yield more information about the distributions of pharmacologically active molecules near the site of administration than of their interaction with membrane receptors.

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References

CURTIS, D.R., DUGGAN, A.W., FELIX, D. & JOHNSTON, G.A.R. (1971a). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord. *Brain Res.*, **32**, 69-96.

CURTIS, D.R., DUGGAN, A.W., FELIX, D., JOHNSTON, G.A.R. & McLENNAN, H. (1971b). Antagonism between bicuculline and GABA in the cat brain. *Brain Res.*, **33**, 57-73.

CURTIS, D.R. & JOHNSTON, G.A.R. (1974). Amino acid transmitters in the mammalian central nervous system. *Ergebn. Physiol.*, **69**, 97-187.

HILL, R.G. & SIMMONDS, M.A. (1973). A method for comparing the potencies of γ-aminobutyric acid antagonists on single cortical neurones using micro-iontophoretic techniques. *Br. J. Pharmac.*, **48**, 1-11.

HILL, R.G., SIMMONDS, M.A. & STRAUGHAN, D.W. (1972). Convulsive properties of d-tubocurarine and cortical inhibition. *Nature, Lond.*, **240**, 51-52.

HILL, R.G., SIMMONDS, M.A. & STRAUGHAN, D.W. (1973a). Amino acid antagonists and the depression of cuneate neurones by γ-aminobutyric acid (GABA) and glycine. *Br. J. Pharmac.*, **47**, 642-643P.

HILL, R.G., SIMMONDS, M.A. & STRAUGHAN, D.W. (1973b). A comparative study of some convulsant substances as γ-aminobutyric acid antagonists in the feline cerebral cortex. *Br. J. Pharmac.*, **49**, 37-51.

JOHNSTON, G.A.R., BEART, P.M., CURTIS, D.R., GAME, C.J.A., McCULLOCH, R.M. & MACLACHLAN, R.M. (1972). Bicuculline methochloride as a GABA antagonist. *Nature, New Biol.*, **240**, 219-220.

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