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Amino-acid induced depression of cortical neurones

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Summary

1. The effects of strychnine on the degree of depression of neuronal firing induced by glycine, γ-aminobutyric acid (GABA) and 5-hydroxytryptamine (5-HT) have been examined quantitatively. All drugs were applied by iontophoresis to spontaneously active cerebral cortical neurones in the anaesthetized cat. The application of these drugs was continued until a plateau or equilibrium depression was reached. The time taken to reach this steady state was noted. Dose-response curves were then constructed for those currents giving less than complete depression.

2. Glycine was less potent than GABA and about 7-fold larger currents were needed to achieve comparable depression. 5-HT was also a weak depressant compared with GABA and had 0·6 the potency of glycine on a current basis.

3. Strychnine in currents up to 25 nA shifted the dose-response curve of glycine to the right at a time when equilibrium depression in the same cells induced by the control agonists GABA or 5-HT was unaffected. These currents of strychnine did, however, prolong the time-course of onset of GABA and 5-HT depression.

4. In larger currents strychnine reduced GABA equilibrium depression, but the dose-response curve was not shifted in a parallel fashion.

5. It is concluded that strychnine can specifically and competitively antagonize the effect of glycine on cortical neurones.

Introduction

Glycine and γ-aminobutyric acid (GABA) depress the rate of firing of feline cortical neurones, and there is evidence that strychnine selectively antagonizes the effect of glycine (Curtis, Hösli & Johnston, 1968). In view of the reports that strychnine also prevents the depression of cortical neurones by acetylcholine (ACh), noradrenaline (NA), 5-hydroxytryptamine (5-HT) and histamine, by Phillis & York (1967) and Phillis, Tebēcis & York (1968), the specificity of strychnine antagonism on cortical neurones must be questioned.

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In the present paper, the action of strychnine on amino-acid and 5-HT depression is examined quantitatively in order to study both the magnitude and time-course of cortical neuronal depression. The results suggest that strychnine behaves as a specific glycine antagonist.

Methods

Details of the techniques used for the extracellular iontophoresis of drugs onto spontaneously firing neurones in the cat posterior sigmoid gyrus have been presented elsewhere (Roberts & Straughan, 1967; Johnson, Roberts & Straughan, 1969b). Anaesthesia was induced by N_2O-halothane and was maintained either on 75% nitrous oxide in oxygen and 1% halothane, or on intravenous pentobarbitone sodium (25 mg initially, supplemented as necessary).

The following procedures were used in the present agonist-antagonist studies:

(*a*) Only spontaneously active neurones were studied as in our previous studies. The population of otherwise quiescent neurones which can be excited by glutamate was avoided (Johnson, Roberts, Sobieszek & Straughan, 1969a).

(*b*) The degree of depression of neuronal firing was measured at the point where continuation of drug application led to no further reduction in firing rate ("equilibrium or plateau response"). The inhibition of firing was then calculated as a percentage, taking zero firing as 100% inhibition and the mean rate of firing before drug application as zero inhibition. The time taken to the equilibrium response was also noted.

(*c*) Reproducible depressant responses were obtained to glycine and either GABA or 5-HT in a regular cycle with at least two different doses of each compound.

(*d*) Strychnine was then applied continuously and the neurone was re-tested as in (*c*).

(*e*) Strychnine was discontinued and recovery of the agonist effects was recorded.

Barbiturate anaesthetized cats were used in experiments with 5-HT to achieve mainly depressant responses (Johnson *et al.*, 1969b)

The following drugs were used in aqueous solution in the micropipettes:

Glycine and γ-aminobutyric acid (GABA) 0·2 M, acidified to pH 3·5 with HCl; 5-hydroxytryptamine hydrogen maleate (5-HT) 0·2 M, pH 3·5; acetylcholine chloride (ACh) 0·2 M, pH 3·5–4·0 and strychnine sulphate 0·01 M, pH 4·0.

Results

GABA depression

GABA was depressant in all cells tested. The response began after a very short latency (usually less than 1 s) and reached an equilibrium (plateau) level after a time which varied between cells and depended on the current of application. Maximal depression (100%) was usually achieved by currents exceeding 12·5 nA and the rate at which this response was obtained was inversely related to the current (Table 1). On occasions, currents as low as 2·5 nA caused complete depression—these cells were not used for antagonism studies. Lower currents of GABA up to

12·5 nA usually gave equilibrium responses without complete (100%) depression (Table 1). Only these submaximal responses were used to construct the dose-response curves.

These equilibrium levels of submaximal depression presumably occur when the concentration of drug in the neuronal environment is relatively constant and the rate of ejection of drug balances with its rate of removal from the tissue.

Glycine depression

Glycine depressed firing in 75% of the neurones tested and had no effect on 25%. If the glycine current was applied for many minutes to a cell initially classified as unaffected, a very gradual increase in the firing rate was occasionally seen. In view of the extremely long time-course of this action, these cells have not been classified as excited. Depression began after an average latency of 4 seconds. In the majority of neurones even currents of glycine as large as 300 nA did not produce 100% depression. Glycine was a weaker depressant of cortical neurones than GABA, with a glycine/GABA current ratio varying between 2 and 20 (mean 7). On cessation of the glycine, the neuronal firing rate recovered to the control level within 5–10 seconds.

5-hydroxytryptamine depression

The characteristics of 5-HT depression of cortical neurones have been described in detail in a previous paper (Johnson *et al.*, 1969a). 5-HT was a much weaker depressant than GABA and slightly weaker than glycine, so that a glycine/5-HT current ratio of 0·6 was needed to produce comparable degrees of depression.

Influence of firing rate changes on magnitude of inhibition

The ideal experimental situation is that in which a spontaneously firing neurone maintains a constant basal discharge rate on which the effects of depressant drugs can be superimposed. However, during prolonged study there is often a significant

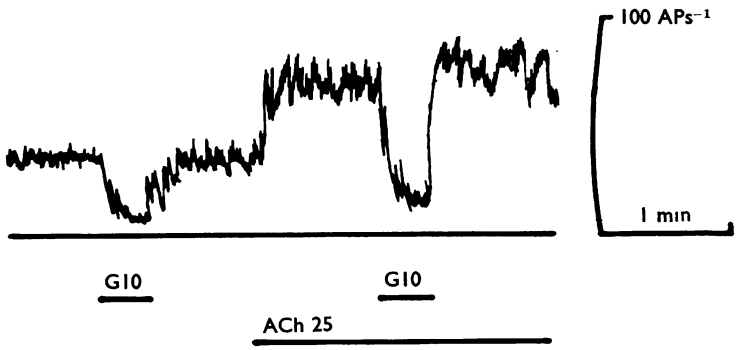

FIG. 1. Ratemeter recording of a spontaneously active cortical neurone from a halothane anaesthetized cat. The neurone firing at 37·5 action potentials (AP) s^{-1} was depressed to 6 AP s^{-1} by 10 nA GABA (G 10)—that is 84% inhibition of firing. The neuronal firing was increased by ACh (25 nA) to 70 AP s^{-1} and again GABA was applied. Firing was then depressed to 12 AP s^{-1}. Thus, the magnitude of depression (% maximal) is independent of the rate of neuronal discharge before drug application.

change in basal firing rate, particularly if excitant drugs are applied. It was necessary, therefore, to establish whether these changes in basal firing rate influenced the degree of drug induced depression. In Fig. 1, a cell firing at 37·5 action poten-

TABLE 1

1	2	3	4	5	6
GABA current nA	% inhibition of basal firing rate by GABA (zero firing =100%)	% inhibition of basal firing rate in presence of strychnine (25 nA)	Time (s) to reach GABA equilibrium response	Time (s) to reach GABA equilibrium response in presence of strychnine 25 nA	Time to GABA equilibrium with strychnine / Control time to equilibrium
2·5	16	15	–	–	–
5·0	62	60	57·0	–	–
10·0	80	76	25·0	63·9	2·5
12·5	90	100	20·0	35·8	1·8
15·0	100	100	17·0	31·0	1·8
20·0	100	100	13·5	27·8	2·0
40·0	100	100	5·1	9·2	1·8
80·0	100	100	1·5	3·5	2·3

The mean experimental values taken from nine neurones demonstrate the lack of effect of strychnine (25 nA) on GABA equilibrium depressant responses (columns 2 and 3), and the prolongation of the GABA time-course by strychnine (columns 4 and 5). Column 6 shows that the ratio of the time to GABA equilibrium in the presence of strychnine to the control time to GABA equilibrium, is relatively constant.

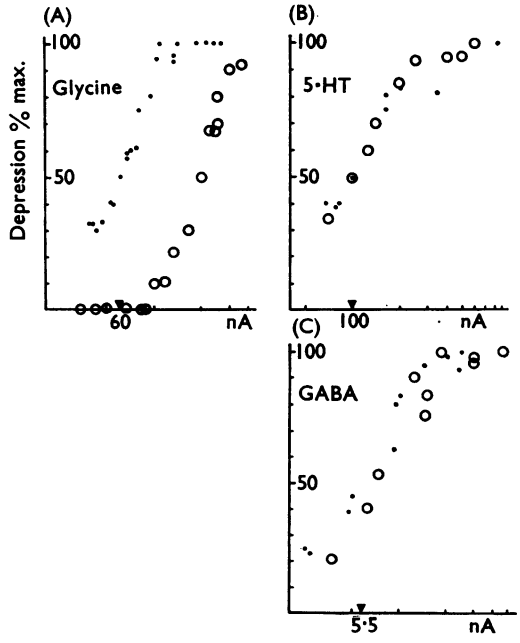

FIG. 2. Log dose (current) response curves to glycine (seven neurones, A) and control agonists 5-HT (four neurones, B) and GABA (three neurones, C), before (●) and during (○) a continuous application of strychnine (5–15 nA). The curves for each drug were superimposed at their 50% response levels. The ordinates are % maximal depression and the abscissae represent current on a log scale. The ED50 is indicated for each drug (▼). It can be seen that responses from different cells superimpose readily and that for any drug the slope of the curve is identical for all cells. The dose-response curve for glycine was displaced by strychnine to the right in a parallel manner, whereas the curves for GABA and 5-HT were unaffected. The mean glycine current ratio (before strychnine : during strychnine) was 1 : 3·4.

tials (AP) s^{-1} was depressed to 6 AP s^{-1} by 10 nA GABA—that is 84% inhibition. The firing rate was then artificially increased by acetylcholine to 70 AP s^{-1} and again 10 nA GABA applied. Firing was then depressed to 12 AP s^{-1}—that is 83% inhibition.

This finding always applied whether the basal firing rate increased spontaneously or in response to the application of ACh. For any neurone, therefore, it seems that a constant dose of depressant agonist will give a constant percentage depression regardless of the firing rate.

Effect of strychnine on neuronal firing

A continuous application of strychnine 25 nA caused a gradual increase in firing rate of all neurones studied. When the increase in firing had reached an equilibrium level (after 5 min), the rate was double the basal spontaneous rate (mean 207·5% ±13·0% S.E.). Increase in the strychnine current beyond 50 nA eventually depressed neuronal firing (Johnson, Roberts & Straughan, unpublished observations). Strychnine currents of less than 20 nA often had no effect on neuronal firing rate.

Specificity of strychnine antagonism

It has been reported that strychnine applied to cortical neurones blocked the inhibitory actions of NA, 5-HT and ACh (Phillis & York, 1967) and histamine (Phillis *et al.*, 1968). However, Johnson, Roberts & Straughan (unpublished observa-

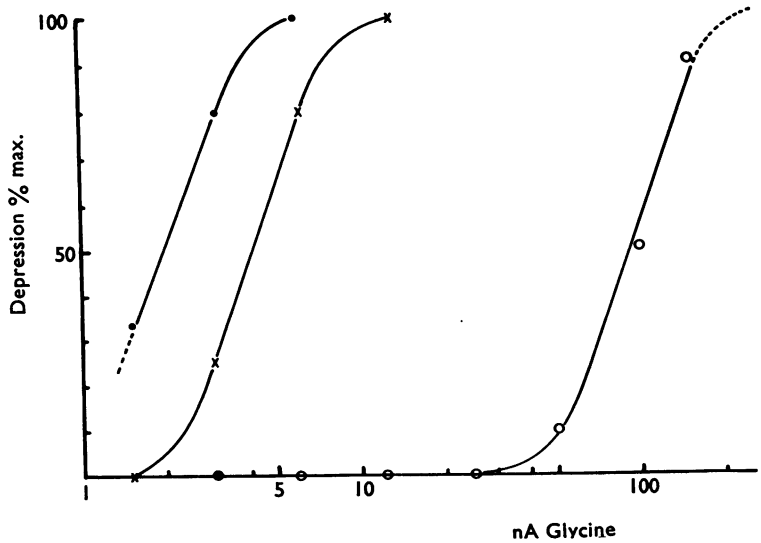

FIG. 3. Log current (dose) response curves to glycine obtained on a single cortical neurone in a halothane anaesthetized cat. The control responses are indicated by the small dots (●) and those in the presence of strychnine 5 nA by the open circles (○). The crosses indicate the recovery of the responses 5 min after the strychnine was discontinued. The curves were fitted visually. Note: (1) the extreme sensitivity of the cell to glycine; (2) the marked parallel displacement to the right by the small current of strychnine (dose ratio 67); (3) the reversible nature of strychnine antagonism. The GABA dose-response curve elicited on the same cell was not affected by strychnine.

tions) have shown that NA, 5-HT and GABA equilibrium depressions were not antagonized by strychnine (25 nA). The apparent reduction of the responses they obtained resulted from the prolongation of the time-course of depression. This effect was most clearly seen when the responses were measured at equilibrium. Table 1 shows that while strychnine (25 nA) had no effect on the percentage inhibition of firing rate by GABA at equilibrium, it did alter the time-course of depression. Glycine was affected quite differently, however, and even small currents of strychnine (5–15 nA) antagonized specifically and reversibly the equilibrium depression by glycine. The results from seven neurones whose dose-response curves were superimposed at their 50% response levels are presented in Fig. 2A. In three of these, GABA was the control agonist (Fig. 2C) and in the remaining four, 5-HT was the control (Fig. 2B). The response curves for glycine (A) were displaced by strychnine to the right in a parallel manner, whereas the curves for GABA (C) and 5-HT (B) were unaffected. The glycine current (dose) ratio (before strychnine: during strychnine) was 1 : 3·4. In a single experiment (Fig. 3) the effect of strychnine was so strong that an almost 70-fold increase in the current applying glycine was needed to produce depressant responses comparable with the control.

Larger currents of strychnine (50 to 100 nA) were required to antagonize GABA equilibrium depressions (Fig. 4). The non-parallel depression of the curve in this figure and several other preliminary experiments may indicate a non-competitive antagonism of GABA effects by strychnine. It is quite likely that this effect on GABA depression is non-specific, as it is seen only with higher and directly depressant currents of strychnine which also affect the depression produced by structurally diverse compounds.

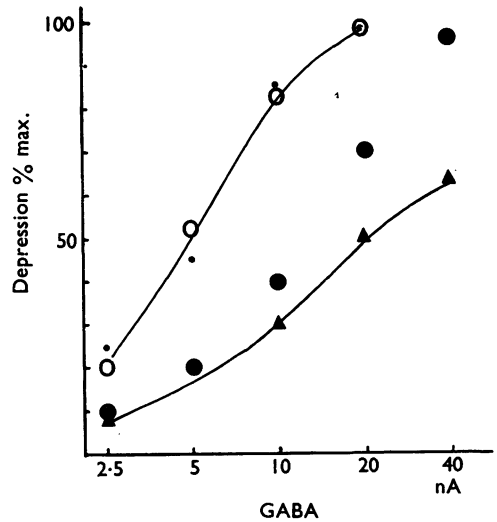

FIG. 4. Log current (dose) response curves to GABA obtained on a single cortical neurone in a halothane anaesthetized cat. The control responses are indicated by the small dots (●) and the curves in the presence of strychnine 25 nA by the open circles (○), strychnine 50 nA by the large filled circles (●) and strychnine 100 nA by the filled triangles (▲). It can be seen that no significant displacement of the dose-response curve occurred with less than 50 nA strychnine. The non-parallel displacement of the curve in the presence of 100 nA strychnine may indicate a non-competitive mode of antagonism.

Action of strychnine on the time taken by GABA to reach equilibrium response

The equilibrium level of depression and the time taken to reach equilibrium are both dependent on the iontophoretic current applying the drug (Table 1). Although a continuous application of strychnine (25 nA) did not affect the equilibrium response to GABA, it prolonged the time taken to achieve that response twofold (Table 1). By measuring the level of response at equilibrium, rather than the depression achieved after any preselected time, the possibility of interpreting prolongation of GABA time-course by strychnine as antagonism is avoided. That the prolongation of GABA time-course was a non-specific effect was evident from the finding that a continuous application of a non-depressant current of glycine also prolonged the GABA equilibrium. The increase in time-course was unrelated to the change in background firing caused by strychnine or glycine.

Discussion

The present quantitative study has shown that strychnine is a specific antagonist of the depressant actions of glycine on feline cortical neurones. With currents of application that did not affect the equilibrium responses to GABA or 5-HT, strychnine displaced the glycine dose-response curve to the right in a parallel manner, giving a current ratio of 3·4. This evidence is consistent with the view that strychnine is a competitive glycine antagonist. The selective nature of strychnine's action is in general agreement with the finding of Curtis *et al.* (1968) on cortical neurones, but contrary to the results of Davidoff, Aprison & Werman (1969), who claim that strychnine is a non-specific non-competitive antagonist of amino-acid depression of spinal interneurones. This discrepancy might possibly be due to differences in the pharmacological actions of strychnine in the cortex and the cord. Davidoff *et al.* (1969) also used cells driven by the excitant amino-acid glutamate. The present study used only spontaneously active neurones, as it has been shown that glutamate can distort the responses of neurones to NA and 5-HT (Roberts & Straughan, 1967; Johnson *et al.*, 1969a). It does not appear to be necesary to use glutamate to achieve a wider response range with the depressant agonist as the present results show that the depressant equilibrium response (% maximal) does not depend on the initial rate of neuronal discharge, whether this be spontaneous or drug-induced.

Davidoff *et al.* (1969) took care to measure their depressant agonist responses at equilibrium. This is essential for any agonist-antagonist study. The present experiments show that a continuous application of strychnine will prolong the time-course of a GABA depressant response twofold, without affecting the response magnitude at equilibrium. This means that the response to a short application of GABA will appear to be reduced by strychnine when no real antagonism has occurred. Johnson, Roberts & Straughan (unpublished observations), in agreement with the findings of Phillis & York (1967), showed that an apparent antagonism of 5-HT, NA and GABA by strychnine occurred when agonists were applied for short intervals, but, as can be seen from the present results, no antagonism of monoamine or GABA equilibrium depression need occur with currents of strychnine that do antagonize the depressant effects of glycine. Larger currents of strychnine (about 50 nA) can reduce the equilibrium response to GABA, but this effect does not appear to be specific.

The nature of the prolongation of the time-course of GABA depression by strychnine is not known. A similar effect was obtained with continuous non-depressant currents of glycine. The increase in the time-course of GABA depression by strychnine was not paralleled by the increase in background firing caused by strychnine.

The present results show that glycine acts on a receptor pharmacologically distinct from that on which GABA and the monoamines act. Strychnine clearly differentiates between glycine and GABA induced depression of the neuronal firing in the cortex as it does elsewhere, for example in the brain stem, Deiters' nucleus and spinal cord (Hösli, Tebēcis & Filias, 1969; Bruggencate & Engberg, 1969; Curtis *et al.*, 1968). The possibility still remains to be excluded that monoamine depressions in the cortex occur indirectly through the release of GABA.

The physiological significance of the glycine receptor on cortical neurones is uncertain. Glycine is unlikely to be a synaptic transmitter as the conductance changes induced by glycine in cortical neurones differ from those produced by the natural inhibitory transmitter and by GABA (Kelly & Krnjević, 1969). However, a modulator role (Florey, 1967) for glycine in the cortex remains to be excluded. Though glycine is a less potent depressant than GABA in the cortex, the absolute levels of glycine are still relatively high; also the extra-neuronal levels of glycine might well be higher than those of GABA to judge from the comparatively high efflux of glycine from the cortical surface (Randić & Straughan, unpublished observations). Strychnine could be expected to block a background depression from glycine and a release excitation would result. Alternatively strychnine could excite neurones directly, but true neuronal disinhibition seems unlikely as most cortical inhibitions are not blocked by strychnine (Krnjević, Randić & Straughan, 1966).

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