

Drug-induced changes in the electrically evoked release of ^3H - γ -aminobutyric acid (^3H -GABA) from spinal cord

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It has been suggested (Eccles, Schmidt & Willis, 1963) that GABA is the transmitter involved in the phenomenon of presynaptic inhibition in the spinal cord and recent evidence, both neurophysiological (Davidoff, 1972) and biochemical (Mitchell & Roberts, 1972), supports this hypothesis. In the present study, the effect of various procedures on the electrically evoked release of ^3H -GABA from frog spinal cords have been investigated.

Sagittally hemisected frog spinal cords were transferred to a 500 μl capacity bath (Mitchell & Phillis, 1962) containing oxygenated amphibian Ringer solution at 10–12° C to which amino-oxyacetic acid (10^{-5}M) had been added to inhibit GABA metabolism. Preparations were incubated for 1 h with ^3H -GABA (10^{-6}M) after which the Ringer solution was replaced at 5 min intervals with fresh solution and the radioactivity in each sample estimated. Using a variety of parameters, electrical stimulation of roots failed to evoke increased GABA release whereas stimulation of the rostral end of the cord caused large consistent increases in the efflux of ^3H -GABA; two release maxima were observed, one at 10 Hz ($132 \pm 14\%$ S.E. of mean of pre-stimulation release, 10 experiments) and a second at 30 Hz ($124 \pm 17\%$ of pre-stimulation release, 10 experiments). Stimulation of the caudal end failed to evoke a significantly increased efflux of ^3H -GABA (5 experiments) and in addition, successive sectionings of the cord from caudal toward rostral end resulted in a progressive diminution in the amount of ^3H -GABA released by electrical stimulation.

TABLE 1. *Effect of various drugs on the electrically evoked release of ^3H -GABA from the intact frog spinal cord*

| Drug | % Increase in GABA release | | % Control | |
|--|----------------------------|--------------------|-----------|-------|
| | 10 Hz | 30 Hz | 10 Hz | 30 Hz |
| Control | 132 ± 14 (10) | 124 ± 17 (10) | — | — |
| p-Hydroxymercuribenzoate (0.01 mM) | $30 \pm 7^*$ (4) | $55 \pm 16^*$ (5) | 23 | 53 |
| Ouabain (0.01 mM) | $44 \pm 9^*$ (4) | $18 \pm 3^*$ (4) | 33 | 15 |
| L-2,4-diaminobutyric acid (1.0 mM) | 169 ± 25 (7) | — | 128 | — |
| Picrotoxin (0.1 mM) | $68 \pm 14^*$ (8) | $41 \pm 7^*$ (5) | 52 | 33 |
| Strychnine (0.1 mM) | — | 116 ± 28 (5) | — | 94 |
| Bicuculline (0.1 mM) | $217 \pm 14^*$ (8) | $188 \pm 8^*$ (12) | 164 | 152 |
| Bicuculline (0.01 mM) | 114 ± 17 (5) | 144 ± 28 (5) | 86 | 116 |
| Minus Ca^{++} | $47 \pm 9^*$ (10) | $52 \pm 12^*$ (10) | 36 | 42 |
| Minus Ca^{++} , plus Mg^{++} | $13 \pm 3^*$ (6) | $13 \pm 3^*$ (6) | 10 | 11 |

The descending tracts of each cord were stimulated electrically (4 min., 1 ms, 5 mA) twice before and twice during drug application and the percentage increase in ^3H -GABA release calculated. The mean of each pair of estimates was used in the calculation of the values in the Table which are the means \pm S.E.M. for the number of preparations shown in parentheses. Asterisk denotes $P < 0.05$.

Table 1 shows the effects of various procedures on the electrically evoked release of ^3H -GABA. Release was calcium dependent and was inhibited by the metabolic inhibitors p-hydroxymercuribenzoate, ouabain and also by picrotoxin. Bicuculline significantly increased evoked release (see Table 1) whereas strychnine was without effect. The differences in the effects of bicuculline and picrotoxin on ^3H -GABA release suggest that blockade of GABA receptors is not their sole mechanism of action.

This work was supported by a grant from the M.R.C. to Professor D. W. Straughan.

REFERENCES

- DAVIDOFF, R. A. (1972). The effects of bicuculline on the isolated spinal cord of the frog. *Exp. Neurol.*, **35**, 179–193.
 ECCLES, J. C., SCHMIDT, R. & WILLIS, W. D. (1963). Pharmacological studies on presynaptic inhibition. *J. Physiol., Lond.*, **168**, 500–530.
 MITCHELL, J. F. & PHILLIS, J. W. (1962). Cholinergic transmission in the frog spinal cord. *Br. J. Pharmac. Chemother.*, **19**, 534–542.
 MITCHELL, J. F. & ROBERTS, P. J. (1972). Evoked release of amino acids from the intact spinal cord. *Br. J. Pharmac.*, **45**, 175–176P.