Effect of bicuculline and strychnine on the acetylcholine content of the amphibian spinal cord

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Strychnine is reported as antagonizing glycine mediated inhibition in the spinal cord ; conversely, bicuculline is reported to abolish GABA-induced inhibition (Curtis *et al.*, 1971a and 1971b). Very little is known, however, about the effect that the absence of such inhibition may have on the spinal acetylcholine (ACh) content.

Experiments were carried out during the months of November and December. After injecting a convulsant frog's (*Rana esculenta*) were decerebrated and the dorsal surface of the spinal cord was exposed. The spinal column was then placed in a cold dish containing oxygenated Ringer solution (NaCl, 115 mM ; KCl, 2 mM ; $CaCl_2$, 1·8 mM). The spinal cord was gently freed from the vertebral canal and its whole ACh content was extracted according to the method of Smallman & Fisher (1958), and bioassayed on the dorsal muscle of the leech.

Drugs dissolved in saline were administered subcutaneously at the doses and times stated in Table 1 which shows that only strychnine increases ACh levels 4 h after administration.

TABLE 1. *Effect of bicuculline and strychnine on the acetylcholine content of the frog spinal cord. (2 spinal cords were pooled in each experiment.)*

Drug	Dose mg/kg s.c.	Number of experiments	Time after administration	ACh μg/g± S.E.	Remarks
None	—	11	—	4·79± 0·40	—
Saline	—	4	30 min	4·60± 0·60	—
Bicuculline	5·0	9	10 min	3·77±° 0·24	Convulsions
Bicuculline	5·0	2	2 h	4·70	,,
Bicuculline	5·0	6	3·5 h	4·26± 0·29	,,
Strychnine	5·0	5	10 min	4·40± 0·39	,,
Strychnine	15·0	4	30 min	4·62± 0·68	Paralysis
Strychnine	5·0	3	4 h	7·72±* 0·39	Convulsions

° Different from controls with $P<0·05$
* Different from controls with $P<0·001$

During this period of time the frogs showed sustained convulsions. Conversely, bicuculline in doses which always produced convulsions produces a decrease in ACh content only 10 min after its injection. Two to 3·5 h after the administration of bicuculline the ACh content returns to the control level. Our data suggest that these two convulsants, strychnine and bicuculline, have opposite effects on spinal ACh levels. Further investigation is necessary to elucidate the exact nature of these effects.

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The effects of gamma-hydroxybutyric acid on brain respiration *in vitro*

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Gamma-hydroxybutyric acid (GHB) has recently been shown to have specific actions on cerebral glucose metabolism, including the ability to stimulate the respiratory rate of rat cerebral cortical slices incubated *in vitro* with glucose as substrate (Taberner, Rick & Kerkut, 1972). This effect is in direct contrast to the effects of other central depressant drugs which, at equivalent concentrations, depress the respiration of brain tissue *in vitro* (McIlwain, 1966). Ahmed & Scholefield (1961) however, have reported that several short chain fatty acids can produce a temporary stimulation of the respiration of brain tissue *in vitro*, but that it is followed by an irreversible inhibition within 2 h. The present experiments were performed further to characterize this singular action of GHB on brain respiration.

Rat cerebral cortical slices were incubated in Krebs-Ringer phosphate medium (pH 7·4) at 37° under air with added substrate (10 mM). With glucose as substrate, the addition of GHB to the medium at concentrations of 0·5–4·0 mM produced a 25% increase in the respiratory rate which was maintained for at least 3 h, although GHB itself did not support respiration. As has been shown earlier, the effect could not be obtained with cerebral cortical homogenates, or with sliced homogenates of rat liver (Taberner *et al.*, 1972). Also the effect was not observed with glucose-6-phosphate, fructose, succinate, oxaloacetate, pyruvate or glutamate as the exogenous substrate. With β-hydroxybutyrate as substrate there was a very marked inhibition of respiration with GHB above 5 mM in the medium. At high concentrations of GHB (above 50 mM), the increase in respiration with glucose as substrate was no longer obtained and the respiration tended to be depressed.

Other central depressant drugs tested, including pentobarbitone and imidazoleacetic acid, invariably depressed the respiratory rate at concentrations above 0·1 mM. Oxidized glutathione which, like GHB, increases the activity of the pentose phosphate pathway in the brain (Hotta, 1962 ; Taberner *et al.*, 1972) also stimulated the rate of respiration of the tissue when glucose was the substrate. However, the effect was not as consistent as that observed with GHB, and it was not obtained with reduced glutathione.

It is confirmed that GHB, unlike other central depressant drugs, increases the rate of respiration of cerebral cortical slices *in vitro*. Since this effect could only be obtained with slices of cerebral cortex respiring with glucose as the exogenous substrate, it is possible that GHB acts by facilitating the entry of glucose into the intact cells.

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