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The effect of 40 mM potassium and electrical stimulation on the efflux of [^{3}H]-GABA from rat dorsal medulla *in vivo* and *in vitro*

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There have been two independent reports that 40 mM potassium causes an increase in the efflux of γ-aminobutyric acid (GABA) from the rat cuneate nucleus *in vivo* (Roberts, 1974; Assumpcao, Bernardi, Dacke & Davidson, 1977). We have also investigated the efflux of [^{3}H]-GABA from that part of the dorsal medulla containing the cuneate nucleus and our experiments do not support the conclusions of these previous workers.

Rats were anaesthetized with urethane (1.25 g/kg), the dorsal surface of the medulla was exposed and an incubation cup formed by placing a small length of tubing (3 mm internal diameter) on the pial surface and sealing it in place with silicone grease. Twenty µl of a Krebs solution containing 6.6×10^{-6} M [^{3}H]-GABA and 1.2×10^{-4} M [^{14}C]-sucrose as a spacemarker (2.8×10^6 dpm each) was placed in the cup for 30–60 min. Normal Krebs solution was then superfused over the surface at a rate of 1 ml per 10 min for 30–120 min after which a change was made to an isotonic solution containing 40 mM potassium. The radioactivity in each 10 min collection was counted after adding 5 ml Instagel (Packard) and a few drops of formic acid and quenching was estimated from the external standard channels ratio. Although multiphasic ^{3}H efflux curves were observed with small deflections immediately following the change-over to high potassium this only occurred in 5 out of 15 experiments and was always accompanied by a corresponding increase in ^{14}C efflux.

The efflux of [^{3}H]-GABA from 0.4 mm slices of rat dorsal medulla was studied *in vitro* as described for rat cerebral cortex slices by Srinivasan, Neal & Mitchell (1969). The radioactivity in each 2 min collection (1–1.5 ml) was estimated as before and the efflux was followed for 40 min before changing to an isotonic solution containing 40 mM potassium. In all 8 experiments no deflections were observed. In contrast, 40 mM potassium caused a large increase in efflux of ^{3}H from rat cortical slices similar to that described by Srinivasan *et al.* (1969). In 6 experiments with slices from rabbit dorsal medulla 40 mM potassium again failed to alter ^{3}H washout. The efflux of ^{3}H from medulla slices could be greatly increased by electrical stimulation (rectangular, 5 msec, 20 mA pulses; 60/sec for 30 s in every 2 min). This stimulation did not alter the efflux of ^{14}C and the effect on ^{3}H could be prevented by previous exposure to high potassium.

In view of these results it is suggested that any small changes in the efflux of ^{3}H from the rat cuneate nucleus *in vivo* corresponding to the superfusion of high potassium solutions may be artifactual, for instance due to shrinkage of the extracellular space (Bourke & Tower, 1966; Roberts, 1976). However the specific electrically evoked release of [^{3}H]-GABA from slices of rat and rabbit dorsal medulla lends support to the hypothesis that GABA may be a transmitter at this site.

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t-Butyl bicyclo phosphate: a convulsant and GABA antagonist more potent than bicuculline

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Bicuculline is now widely accepted as a selective GABA antagonist (Curtis & Johnston, 1974) and is at present the most potent on mammalian systems. We previously studied a series of convulsant compounds of the formula 4(R)-1-phospha 2,6,7 trioxabicyclo (2,2,2) octane-1-oxide (R-PTBO where R = alkyl group) and showed that the isopropyl derivative (IPTBO) was equipotent with bicuculline as a GABA antagonist (Bowery, Collins & Hill, 1967a; Bowery, Collins, Hill & Pearson, 1976b). Other alkyl substituted PTBO derivatives have now been studied, n-propyl (n-ProPTBO), n-butyl (nBPTBO), s-butyl (s-BPTBO), t-butyl (t-BPTBO), methyl (MPTBO) and pentyl (PPTBO), and our results indicate that the t-BPTBO derivative is more potent than bicuculline.

Convulsant potency was determined by intravenous injection in adult mice. GABA antagonism was assessed from the depression of depolarizing responses to GABA in the frog spinal cord and rat superior cervical ganglion as described previously (Bowery *et al.*, 1976b). Relative potencies were obtained by comparison with IPTBO and bicuculline at concentrations required to inhibit responses to fixed submaximal doses of GABA by 50%. This method was adopted since previous experiments have indicated that the PTBO derivatives appear to act non-competitively (Bowery *et al.*, 1976a).

All the derivatives had similar actions to those already described for IPTBO (Bowery *et al.*, 1976a, 1976b). Convulsions consisted of rapid clonic jerks leading to tonic extension with larger doses. CD_{100} values are shown in Table 1. Responses to GABA (0.5–4 mM) in the frog spinal cord were readily antagonized by the PTBO derivatives whereas response to glycine and glutamate were unaffected. The derivatives also antagonized the depolarizing action of GABA (1–300 μM) in the superior cervical ganglion without affecting responses to carbachol. The relative molar potencies are shown in Table 1.

Table 1

Compound	CD_{100} μg/kg* i.v. mice	Relative molar potency as GABA antagonist† Frog spinal cord	Rat superior cervical ganglion
t-BPTBO	25	3.5	4.2
IPTBO	140	1.0	1.0
s-BPTBO	100	0.47	1.0
n-ProPTBO	300	0.2	0.2
n-BPTBO	800	0.08	0.07
PPTBO	1200	<0.01	<0.01
MPTBO	>4000	—	<0.01
Bicuculline	200	0.5‡	1.0‡

* Mean values each determined from 8 groups of 4 mice.
† Determined by comparison with IPTBO within the same experiment. Mean values from 2–6 experiments for each compound.
‡ Bicuculline methochloride.