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γ-Aminobutyrylcholine and GABA receptors on primary afferents in the frog spinal cord

There has recently been considerable discussion on the pharmacology of γ-aminobutyrylcholine (GABACh). Although the structural similarities between GABACh and the γ-aminobutyric acid (GABA) antagonists, bicuculline (Howells, 1971) and *N*-methyl bicuculline (Pong & Graham, 1972), suggest that GABACh might intereact with GABA receptors, most pharmacological studies, as reviewed by Johnston & Curtis (1972), have found GABACh to have little GABA-like activity (Honour & McLennan, 1960; Hance, Winters & others 1963; Curtis, Phillis, & Watkins, 1961; Krnjević, 1964; Crawford & Curtis, 1964). Bowery & Brown (1972) tested GABACh on sympathetic ganglia which possess both acetylcholine and GABA receptors and found that GABACh has little acetylcholine-like activity but strong GABA-like activity. Since the GABA-like activity was blocked by cholinesterase inhibitors, these investigators concluded that the GABA-like activity principally results from the formation of free GABA by the hydrolysis of GABACh. I have noted a less potent GABA-like activity of GABACh on primary afferent fibres which is entirely resistant to cholinesterase inhibitors. Thus these results suggest that GABACh can interact with the GABA receptors on primary afferents or that, if hydrolysis occurs, the enzyme involved is resistant to cholinesterase inhibitors (cf. Curtis & others, 1961; Holmstedt & Sjöqvist, 1960).

The effect of drugs on the membrane potential of primary afferent fibres in the frog isolated spinal cord (*Rana pipiens*) was measured by sucrose gap recording (Barker, Nicoll & Padjen, 1975). The Ringer solution contained either 20 mM $MgSO_4$ or 1 μM tetrodotoxin to block indirect synaptic effects. All experiments were at room temperature (20°). Thin-layer chromatography demonstrated that the GABACh contained no free GABA. Butanol–acetic acid–water (200 : 30 : 75, by vol.) was used as solvent. The chromatographs were exposed to iodine vapours for visualizing the spots. The GABACh was prepared in Ringer solution at the beginning of each experiment, to minimize the possibility of spontaneous hydrolysis.

In all 15 preparations GABACh exerted a depolarizing action on the primary afferents. The action of GABACh and GABA, unlike that of acetylcholine and carbachol, was not blocked in the presence of $MgSO_4$ or tetrodotoxin. The responses in Fig. 1A were obtained in a preparation in which synaptic transmission was blocked with tetrodotoxin. Both GABA and GABACh depolarize the dorsal root, while carbachol, which depolarizes in normal Ringer solutions, has a slight hyperpolarizing action in a Ringer containing 20 mM $MgSO_4$. The GABACh response often lasted up to 10 min after the application, while the GABA response subsided quickly after the application. The potency of GABACh relative to GABA was 0·05. The dose response curves for GABA and GABACh are shown in Fig. 1B.

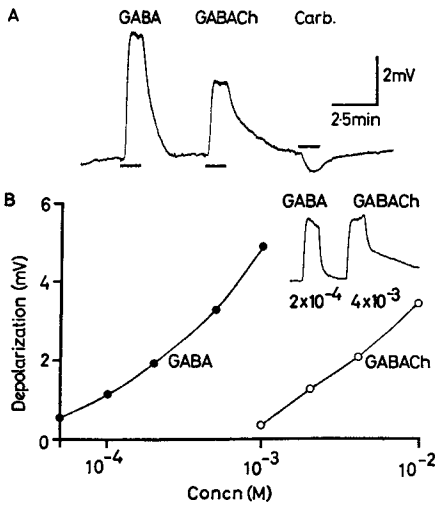

FIG. 1. A comparison of the action of GABA, GABACh and carbachol on primary afferent terminals. A shows the responses to GABA (10^{-3}M), GABACh (10^{-2}M) and carbachol (10^{-3}M) in a preparation perfused with 10^{-6}M tetrodotoxin to block synaptic transmission. B shows the dose-response curves for GABA and GABACh. Each point represents the average of 5 experiments. The inset shows equipotent concentration of GABA and GABACh.

The observations that $MgSO_4$ and tetrodotoxin block the depolarizing action of acetylcholine-like agents but not the action of GABACh on primary afferents suggests that GABACh is not exerting its effect through cholinergic receptors. The use of a number of acetylcholine and amino acid antagonists established that the depolarization elicited by GABACh occurred through the activation of GABA receptors. Neither atropine (10^{-3}M) nor dihydro-β-erythroidine (5×10^{-4}M) which block carbachol and acetylcholine responses in frog spinal cord (unpublished observations: Phillis & Tebécis, 1967) had any effect on the action of GABACh. Curare did antagonize the action of GABACh (Fig. 2A) but this antagonism was identical to that observed with GABA (unpublished observations). Both picrotoxin and bicuculline (Fig. 2B) antagonized the action of GABACh, while strychnine, at concentrations which blocked the response to β-alanine, failed to block the response to GABACh (Fig. 2C). These results with antagonists indicate that the action of GABA and GABACh are indistinguishable. Could this action of GABACh result from its enzymatic hydrolysis to free GABA (cf. Bowery & Brown, 1972)? To test this possibility the effect of the cholinesterase inhibitors, physostigmine and neostigmine, on the GABACh response were examined. In none of the four preparations tested did these agents (10^{-4}M) affect the GABACh response (Fig. 2D).

These results are in accord with those of Bowery & Brown (1972) on sympathetic ganglia suggesting that GABACh has little acetylcholine-like activity and only weak GABA-like activity. However, the response on primary afferents, unlike that on sympathetic ganglia, is entirely resistant to the action of cholinesterase inhibitors. Thus either GABACh can interact with GABA receptors in this system or the enzyme involved in its hydrolysis is resistant to the action of cholinesterase inhibitors (cf. Holmstedt & Sjöqvist, 1960). It is interesting that GABACh is generally ineffective on central neurons, and this may be due to the limited concentrations obtainable by the iontophoretic technique (i.e., approximately 10^{-3}M, Curtis, 1964), or, alternatively, the GABA receptors on central neurons may differ from those on primary afferents.

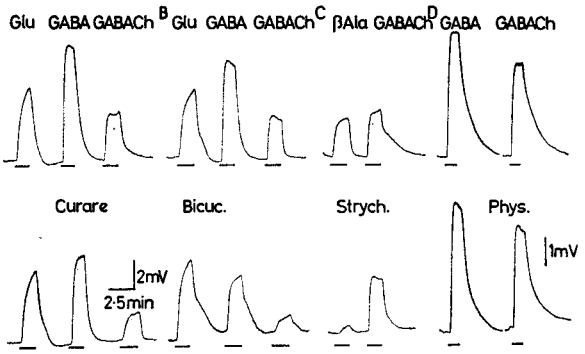

FIG. 2. Pharmacology of GABACh responses. A. Curare (5×10^{-4}M), applied for 10 min, reduced the GABA and GABACh response, but not the glutamate response (Glu). B shows that bicuculline (10^{-4}M), applied for 15 min, also selectively reduces the GABA and GABACh responses. In C strychnine (10^{-4}M), applied for 8 min, reduces the β-alanine (β Ala) response, but not the GABACh response. The application of physostigmine (10^{-4}M) for 10 min (D) has no effect on the GABA or GABACh response. The time calibration in A applies to all records, while the voltage calibration in A applies to B and C. The concentration of all amino acids is 10^{-3}M except GABA which is 10^{-2}M.

It has been found that GABACh does not interact with the uptake sites for GABA (Beart & Johnston, 1973), which could explain the long duration of the GABACh response. Johnston & Curtis (1972) and Bowery & Brown (1972) concluded that GABACh is an unlikely alternative to GABA in bicuculline-sensitive inhibition based on the relative activity of GABACh to GABA. My results on primary afferents agree that GABACh is unlikely to be the transmitter mediating bicuculline sensitive primary afferent depolarization (Davidoff, 1972; Barker, & Nicoll, 1972).

I thank Dr. J. Klingman for his help with the thin-layer chromatography and Dr. Holmstedt, Department of Toxicology, Karolinska Institute, Stockholm, Sweden, for the generous supply of GABACh.

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February 5, 1975

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