γ-Aminobutyrylcholine: actions on GABA and acetylcholine receptors

γ-Aminobutyrylcholine (GABACh), the choline ester of γ-aminobutyric acid (GABA) has recently been the subject of discussion in these columns. Howells (1971) proposed GABACh as a central inhibitory transmitter, as an alternative to GABA. Arguing against this proposal, Johnston & Curtis (1972) pointed out that the depressant actions of GABACh on mammalian central neurons was weak compared with that of GABA, except on spinal Renshaw cells, and suggested that the pharmacological actions of GABACh might be directed toward cholinergic systems rather than those involving GABA.

Some observations we have made on the action of GABACh on sympathetic ganglia seem to have some relevance to this latter suggestion. Sympathetic ganglion cells are depolarized by GABA through an action on receptors which, in terms of agonist and antagonist specificity, closely resembles those mediating GABA-induced hyperpolarization of central neurons (de Groat, 1970; Bowery & Brown, 1972). Since ganglion cells also possess both 'nicotinic' and 'muscarinic' receptors for acetylcholine (Volle, 1966; Brown, 1966b) this tissue is useful for comparing the affinities of GABACh for GABA and acetylcholine receptors.

We have found that GABACh has little acetylcholine-like activity on rat sympathetic ganglia but strong GABA-like activity. The latter appears to result principally from hydrolysis to free GABA by ganglionic cholinesterase.

Depolarization of isolated rat superior cervical ganglia was recorded with surface electrodes as described previously (Brown, 1966a).

GABACh was synthesized from GABA and choline in dioxane as the chloride salt and purified by recrystallization from ethanol-ether solution (British Patent, 1960). The crystals had a melting point of 193°. Paper electrophoresis of the material in aqueous solution (Potter & Murphy, 1967) revealed less than 0·2% free GABA or choline as contaminants.

GABACh depolarized the ganglion at a threshold concentration of 4μM. Comparison with the actions of GABA and carbachol (Fig. 1) showed this to result predominantly from stimulation of GABA receptors. (i) GABACh depolarization was transient, like that produced by GABA but unlike that seen with carbachol. (ii) In the presence of GABA responses to both additional GABA and GABACh were depressed whereas those to carbachol persisted (Fig. 1a). (iii) Effects of GABACh and GABA were not reduced by hyoscine or a combination of hyoscine and hexamethonium which antagonized the actions of carbachol on muscarinic and nicotinic receptors (Fig. 1b). (iv) Bicuculline depressed the effects of GABACh and GABA by equal amounts and to a greater extent than those of carbachol (Fig. 1c).

GABACh was about half as active as GABA itself as a depolarizing agent (molar potency ratio at 50% of the maximal GABA response, 0·45 ± 0·02; mean ± s.e. of mean from 4 ganglia). This response level corresponded to a GABACh concentration of ≈ 30μM. At 10 times higher concentrations some additional effect on acetylcholine receptors could be detected. The depolarization attained then exceeded that produced by GABA itself, was partly resistant to GABA 'desensitization' and partly reduced by hexamethonium. This acetylcholine-like activity of GABACh was estimated to be ≈ 1/100 of that of carbachol.

When ganglionic cholinesterase was inhibited with physostigmine (2 experiments) or neostigmine (1 experiment) the effective depolarizing concentration of GABACh was raised some 50 times (Fig. 2). Under these conditions the depolarizing concentration of acetylcholine is reduced about 100 times (cf. Brown, 1966a), while the effects of GABA and carbachol were unchanged. From this it would seem that

bath-applied GABACh was hydrolysed about half as rapidly as acetylcholine. This is much faster than the rate of hydrolysis suggested by previous measurements on tissue homogenates (Holmstedt & Sjoqvist, 1960; Tabachnick, 1960). The depolarization produced by GABACh after cholinesterase inhibition was still GABA-like rather than acetylcholine-like. This may reflect residual hydrolysis to GABA or the intrinsic action of unhydrolysed GABACh. In the latter case, the true potency of GABACh on ganglionic GABA receptors would be at most 0·01 of that of GABA.

This apparently weak activity of unhydrolysed GABACh on ganglionic GABA receptors accords with observations on most other mammalian neurons (reviewed by Johnston & Curtis, 1972). It might be that the unusually strong action of GABA on the cholinergically-innervated Renshaw cells (Curtis, Phillis & Watkins, 1961), like that on the ganglion cells, results from local hydrolysis to free GABA. This possibility was considered by Curtis & others (1961), but rejected because of GABACh's slow *in vitro* hydrolysis rate, and because anticholinesterase agents did not obviously affect the action of GABACh. However, the nature of the response

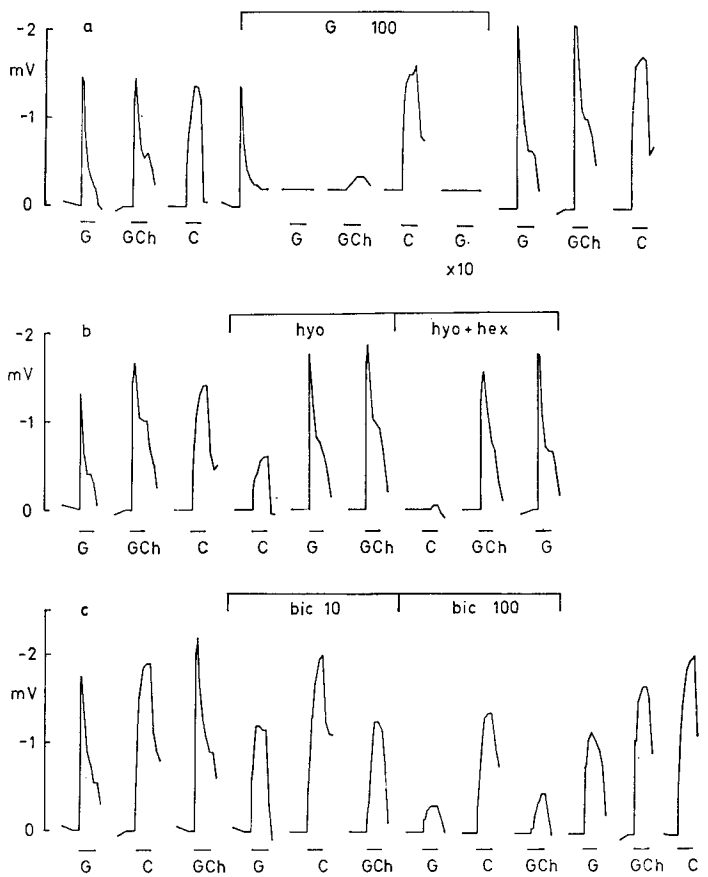

FIG. 1. Depolarization of rat isolated ganglia by GABA (G, 100 μM), GABACh (GCh, 200–250 μM) and carbachol (C, 4·4–5 μM). Ordinates, ganglion depolarization recorded with respect to the postganglionic trunk (mV), the potential difference between the two being reset to zero between drug applications. Abscissae, time, each drug was applied for 4 min (indicated by horizontal bars); breaks in record cover gaps of at least 15 min. Experiments on three separate ganglia show (a) effects of a prolonged application of GABA (b) 2·6 μM hyoscine (hyo) and 2·6 μM hyoscine with 0·83 mM hexamethonium (hex), and (c) 10 and 100 μM bicuculline (bic) on responses to the three depolarizing agents. During prolonged GABA application the test dose of GABA was increased ten-fold ($\times$ 10).

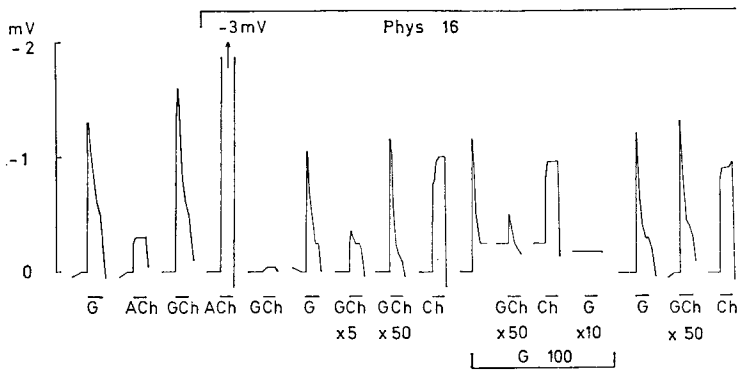

FIG. 2. Effect of 16 μM physostigmine (Phys) on ganglion depolarization produced by GABA (G, 50 μM), GABACh (GCh, 100 μM), acetylcholine (ACh, 55 μM) and choline (Ch, 55 μM). Note that, in the presence of physostigmine, the concentration of GABACh used was increased to 0·5 and 5 mM ($\times 5$ and $\times$ 50). In the presence of 100 μM GABA the response to this increased concentration of GABACh was depressed as in Fig. 1.

described by Curtis & others (1961)—initial depression of both acetylcholine- and glutamate-induced responses, followed by excitation—is very much that to be expected from hydrolysis to free GABA and choline respectively. The alternative explanation of selective acetylcholine receptor block (Johnston & Curtis, 1972) seems less attractive, in view of GABACh's weak effect on ganglionic acetylcholine receptors and those of other tissues such as frog rectus and guinea-pig ileum (Kuriaki, Yakushiji & others, 1958; Holmstedt & Sjoqvist, 1960). The more pronounced blocking effect of GABACh on mammalian neuro-muscular transmission (Asano, Noro & Kuriaki, 1960), which seems to be of the 'depolarizing' type (Holmstedt & Sjoqvist, 1960), could well result from hydrolysis to free choline (cf. Hutter, 1952). Formation of choline might also contribute to the stimulation of ganglionic acetylcholine receptors by GABACh.

Thus, our experiments support the view of Johnston & Curtis (1972) that GABACh is an unpromising alternative to GABA as an inhibitory transmitter. Neither does it seem particularly active on acetylcholine receptors. Its pharmacological activity may however be "related to cholinergic systems" (Johnston & Curtis, 1972) through its apparent susceptibility to hydrolysis by cholinesterase.

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Disodium cromoglycate and the dextran response in rats

Recently, Orr, Hall & others (1971) showed that low concentrations (1×10^{-5}M) of disodium cromoglycate (DSCG) inhibited the release of histamine from rat isolated mast cells induced by compound 48/80. However, Assem & Richter (1971) found that significant inhibition of histamine release by compound 48/80 from rat peritoneal mast cells was effected only with high concentrations (1×10^{-3}M) of DSCG. They also reported that DSCG (100 mg/kg) inhibited the intracutaneous response to dextran in rat skin. We have examined the effects of DSCG on the dextran response in different tissues of the rat.

Groups of five male Sprague-Dawley rats (250–300 g) were injected intraperitoneally with dextran (180 mg/kg, molecular weight 67 000) alone or containing DSCG (dissolved in the dextran solution), and the anaphylactoid response was assessed by a method similar to that of Parratt & West (1957) using an arbitrary scale at half-hourly intervals for 4 h. Other rats received intradermal injections (8/rat) of 0·1 ml Tyrode solution containing either dextran (200 μg) or histamine (36 μg) into the shaved skin of the back, the animals previously having been injected intravenously with azovan blue dye (30 mg/kg); some of the rats also received intravenous injections of DSCG immediately before the intradermal injections. 30 min later, the rats were killed and the amount of blue dye in each wheal was estimated after extraction and assay using the method of Harada, Takeuchi & others (1971).

To study histamine release from the peritoneal cavity, rats were injected intraperitoneally with 1 ml normal saline (0·9%) containing different amounts of DSCG. 30 s later they received an intraperitoneal injection of dextran (180 mg/kg) and heparin (1 mg/kg). 5 min later the animals were killed, the peritoneal fluid collected and centrifuged, and the supernatant was assayed for histamine on the atropinized guinea-pig ileum.

DSCG (5 mg/kg) significantly inhibited the anaphylactoid reaction produced by intraperitoneal dextran, but only after 2 h, the maximum response being at 4 h when the reaction was depressed by about 20%. Doses higher than 5 mg/kg produced less inhibition.

Intravenous DSCG (10 mg/kg) significantly reduced the increased vascular permeability produced by intradermal dextran (as determined by the amount of blue dye leaking into the tissues: dextran 22 $\pm$ 2·5 μg, dextran + DSCG 10 $\pm$ 7 μg), but this dose did not reduce the histamine response. Higher intravenous doses of DSCG produced no further reduction in the response. Intradermal DSCG (36 μg) was also effective in reducing the intradermal dextran response.

Dextran injected intraperitoneally released histamine (range 0·5–1·13 μg/ml) from the peritoneal cavity of rats and intraperitoneal DSCG inhibited this release over the range 2·5–2500 μg/kg (Fig. 1). Thus DSCG inhibits the dextran response in all three tests.

DSCG has been used successfully in the treatment of allergic asthma and as a means of identifying immunological systems that have a common pathway for mediator release. Thus DSCG inhibits degranulation of rat mast cells sensitized with reaginic antibodies on exposure to the antigen (Goose & Blair, 1969). It also prevents mediator release from passively sensitized human lung on exposure to