by addition of Leu-enkephalin. Preliminary results suggest that (−)-baclofen is the active isomer. Neither the response to GABA nor baclofen could be antagonised by bicuculline ($10^{-4}$ M). Similar results were obtained in the gut using the field-stimulated guinea-pig ileum preparation (Kosterlitz & Watt, 1968).

These results suggest that a novel type of GABA receptor, for which baclofen is a potent, selective agonist, may be widespread in peripheral nerve tissues. If this receptor is also present in the brain it may be important in mediating the physiological and therapeutic effects of baclofen.

D.R.H. is an SRC student.

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## Sodium independent GABA receptor binding in peripheral nervous tissue

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The presence of a receptor for γ-aminobutyric acid (GABA) in peripheral sympathetic ganglia is now firmly established (Bowery & Brown, 1974; Adams & Brown, 1975). The structural requirements of agonists for this peripheral GABA receptor are strikingly similar to those of agonists for the GABA receptor mediating hyperpolarisation in the mammalian central nervous system. Recent understanding of the central GABA receptor has been greatly facilitated by studying the specific binding of [$^3$H]-GABA and [$^3$H]-muscimol to membrane fractions (e.g. Zukin, Young & Snyder, 1974; Enna, Beaumont & Yamamura, 1978). We now report that specific binding of these GABA receptor ligands can be detected in sympathetic ganglia.

Calf superior cervical ganglia (individual weight 0.5–0.8 g) were excised immediately after death and maintained at 4°C. Each one was chopped roughly after removal of superficial tissue and homogenised in 10 volumes Tris-citrate buffer (50 mM, pH 7.1) using Ultra-Turrax and glass homogenisers. The combined suspension was centrifuged at 2300 g for 30 min, the pellet washed once with the original volume of buffer and stored at −20°C.

For the binding studies the pellet was routinely resuspended in sodium-free Tris buffer containing Triton-X-100 (0.05% v/v optimal concentration) and incubated for 30 min at 37°C after which the suspension was divided into aliquots containing 50 mg tissue (~2.5 mg protein) and centrifuged at 7500 g for 10 minutes. The resulting pellet was resuspended in Tris buffer to which was added tritiated GABA (66 Ci/mmole, 5 nM) or muscimol (19 Ci/mmole, 5 nM) with or without an excess of unlabelled drug (final volume 1.0 ml). The mixture was incubated for 10 min (by which time binding was maximal) at 4°C or room temperature and the reaction terminated by centrifugation (7500 g, 10 minutes).

The binding of [$^3$H]-GABA or [$^3$H]-muscimol to the pellet was reduced in a dose-dependent manner by the addition of unlabelled GABA ($IC_{50}$ values 1 μM and 0.1 μM respectively). This 'specific' portion of the bound ligand represented 11 ± 1.5% ($n = 11$) and 9 ± 0.5% ($n = 30$) of the total bound [$^3$H]-GABA and [$^3$H]-muscimol respectively. Maximum displacement of bound tritium occurred at 100 μM GABA. Specific binding was also suppressed by the known GABA-mimetics 3-aminopropane sulphonic acid (100 μM), isoguvacine (1 mM) and β-hydroxy GABA (100 μM) and by the GABA antagonist (+)-bicuculline (100 μM). By contrast (−)-bicuculline (1 mM), which is only a very weak GABA antagonist centrally or in sympathetic ganglia, (Collins & Hill, 1974; Bowery, Col-

lins, Cryer, Inch & McLaughlin, 1979) and the GABA transport inhibitors (±)-nipecotic acid (100 μM) and (±)-cis 3-aminocyclohexane carboxylic acid (100 μM) failed to reduce total binding.

Specific binding could be detected in fresh as well as frozen tissue provided that the pellet in both cases was pretreated with Triton-X-100 (cf. Enna & Snyder 1976).

We conclude that the saturable $Na^+$ independent binding of [$^3$H]-GABA and [$^3$H]-muscimol observed in these experiments is associated with the GABA receptor.

D.R.H. is an S.R.C. student.

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## The actions of cimetidine, mepyramine, indomethacin and aprotinin (Trasylol) on the inflammatory response in adjuvant rats

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To study the relative importance of histamine, kinins and prostaglandins in the development of chronic inflammation, the effects of some inhibitors of the actions or formation of these mediators have been evaluated using rats with adjuvant arthritis.

Adjuvant arthritis was induced in male Wistar rats (350–450 g) by a single injection of Freund's complete adjuvant (0.1 ml) into the left hind paw (Newbould, 1963). The acute response was followed for the first 6 h by half-hourly measurement of percentage increase from zero time (D%) of left hind paw volumes, using a Basile differential volume meter. The chronic response was followed for 14 days by daily measurement of the D% of stifle joint lateral diameters using vernier callipers. Drugs were administered 2 h prior to adjuvant injections and thereafter at 24 h intervals. Control groups contained untreated adjuvant injected rats. Results are shown in Table 1.

In the untreated adjuvant controls, all paw volumes increased continuously throughout 6 hours. The mean D% in stifle joint diameters showed a biphasic pattern, peaking at day 4, decreasing until day 6 and increasing again to plateau between days 9–14.

Indomethacin, a cyclooxygenase inhibitor and Trasylol, an inhibitor of kallikrein and similar proteases, produced large reductions in the development of both acute and chronic responses. Thus, both arachidonate metabolites and kinin-forming enzymes or similar proteases were apparently involved in the development of both phases of inflammation. Mepyramine maleate, an $H_1$ antihistamine reduced the D% in joint diameter only between days 1–4, while cimetidine hydrochloride, an $H_2$ antagonist suppressed both the acute and chronic responses at all stages after 3 hours. The presence of $H_2$ but not $H_1$ receptors has been reported in the synovial vasculature in dogs (Grennan, Rooney, St. Onge, Brooks, Zeitlin & Dick, 1975).

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