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Baclofen: a selective agonist for a novel type of GABA receptor

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Recent findings have demonstrated GABA receptors on sympathetic neuroterminals which depress transmitter outflow (Bowery & Hudson, 1979). These receptors are atypical in that they are insensitive to the GABA antagonist bicuculline and to several classical GABA agonists such as 3-aminopropane sulphonic acid (3-APS). Similar findings have been reported on transmitter outflow in sympathetic ganglia (Brown & Higgins, 1979). We now report that baclofen (β(p-chlorophenyl)GABA) is a potent agonist for this receptor.

A range of putative GABA agonists was tested for their ability to depress the evoked release of [^{3}H]-noradrenaline from rat atria as previously described (Bowery & Hudson, 1979) and the contractile response of the field stimulated mouse vas deferens preparation (stimulus parameters after Shaw & Turnbull, 1978).

In both tissues, GABA produced a dose-dependent inhibition of the response (ED_{50} 3–4 μM). Most drugs tested were less potent than on classical GABA receptors such as those mediating depolarisation in the spinal cord or autonomic ganglion.

(±)-Baclofen, however, was equipotent with GABA at inhibiting the response in both tissues (see Table 1). Cross desensitisation occurred in the atrium between GABA and baclofen, but not between GABA and carbachol. Furthermore, contraction of the vas deferens in the presence of a supramaximal concentration of GABA (10^{-4} M) was not further depressed by baclofen, although it could be further inhibited

Table 1 Molar potency ratios of GABA agonists (GABA = 1)

Tissue	*Rat atrium Inhibition of [^{3}H]-noradrenaline release*		*Mouse vas deferens Inhibition of contraction*		*Guinea-pig ileum Inhibition of contraction*		*Rat sympathetic ganglion Depolarisation*	
Drug	ED_{50} (μM)	*Potency Ratio*	ED_{50} (μM)	*Potency Ratio*	ED_{50} (μM)	*Potency Ratio*	ED_{50} (μM)	*Potency Ratio*
GABA	4.2 ± 1.4 ($n = 6$)	1	3.0 ± 0.4 ($n = 12$)	1	7.1 ± 0.5 ($n = 5$)	1	12.5*	1
Muscimol		0.015 ± 0.003 ($n = 3$)		0.13 ± 0.03 ($n = 6$)		0.04 ± 0.005 ($n = 5$)		5.1 ± 0.7**
(±)-Baclofen		0.93 ± 0.02 ($n = 3$)		1.03 ± 0.27 ($n = 6$)		0.97 ± 0.1 ($n = 5$)		≪0.0004*
3-Aminopropane sulphonic acid		≪0.0003 ($n = 3$)		≪0.003 ($n = 3$)				3.4 ± 0.33*
Maximum inhibition (% of control)	54.1 ± 2.34		32 ± 4.9		18 ± 4.2			

* Bowery & Brown (1974).
** Bowery, Collins, Hudson & Neal (1978).
* See also Ault & Evans (1978).

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-