ELSEVIER

European Journal of Pharmacology 329 (1997) 133–136

ejp

Short communication

Actions of thienyl analogs of baclofen at $GABA_B$ receptors in rat neocortical slices

Jennifer Ong [a,*], David I.B. Kerr [a], Claude Vaccher [b], Pascal Berthelot [b]

[a] *Department of Anaesthesia and Intensive Care, The University of Adelaide, Adelaide, South Australia 5005, Australia*

[b] *Laboratoire de Pharmacie Chimique et de Chimie Thérapeutique, Faculté des Sciences, Pharmaceutiques et Biologiques, Université de Lille, BP 83, 59006 Lille Cedex, France*

Received 13 March 1997; revised 29 April 1997; accepted 7 May 1997

Abstract

In rat neocortical slices maintained in Mg^{2+}-free Krebs medium, baclofen and its thienyl analogs, 4-amino-3-(5-chlorothien-2-yl)-butanoic acid (5h), 4-amino-3-(5-methylthien-2-yl)-butanoic acid (5d), 4-amino-3-(5-bromothien-2-yl)-butanoic acid (5f) and 4-amino-3-(thien-3-yl)-butanoic acid (5j) dose-dependently suppressed the spontaneous discharges, antagonised by the $GABA_B$ receptor antagonist 2-hydroxysaclofen (200 μM). Their relative potencies were baclofen > 5h > 5d > 5f > 5j. These heterocyclic analogs may prove useful as $GABA_B$ receptor agonists in functional studies. © 1997 Elsevier Science B.V.

Keywords: Baclofen; $GABA_B$ receptor agonist; Thienyl baclofen analog; 2-Hydroxysaclofen; Neocortical slice, rat

1. Introduction

The neurotransmitter γ-aminobutyric acid (GABA) mediates neuronal inhibition in a variety of synapses in the mammalian central nervous system. It is now recognised that GABA acts at three distinct classes of receptor, distinguished by the bicuculline-sensitive $GABA_A$ receptor which has an integral ligand-gated chloride channel, and bicuculline-insensitive $GABA_B$ and $GABA_C$ receptors (Kerr and Ong, 1995). $GABA_B$ receptors are specifically activated by β-*p*-chlorophenyl-GABA (baclofen), and antagonised by its phosphonic and sulfonic analogs, phaclofen and 2-hydroxysaclofen (Kerr and Ong, 1995). With baclofen, the β-4-chlorophenyl substituent on the GABA backbone imparts the specificity for $GABA_B$ receptors, with marked loss of potency if the chloro-substituent on the phenyl ring is shifted from the 4-position or altered to other halides (Bowery et al., 1981). Although agonist activity is retained in baclofen analogs where the carboxylic moiety is replaced by a phosphinic (Froestl et al., 1995) or sulfinic residue (Carruthers et al., 1995), there have been few studies of the influence of aromaticity at the β-substituent. Recently, in a study of this influence, a series of racemic heterocyclic analogs structurally related to baclofen were synthesised, including β-thienyl and β-furyl derivatives (Berthelot et al., 1991). Of these, the furyl-analogs are inactive in binding studies on $GABA_B$ receptors, but 4-amino-3-(5-methylthien-2-yl)-butanoic acid (5d), and 4-amino-3-(5-bromothien-2-yl)-butanoic acid (5f) as well as 4-amino-3-(5-chlorothien-2-yl)-butanoic acid (5h) (chemical structures shown in Fig. 1) are relatively potent displacers of *R*-(−)-[^{3}H]baclofen (Berthelot et al., 1991). In both the guinea-pig isolated ileum and the spinal cord, these compounds are $GABA_B$ receptor agonists (Ong et al., 1992a; Lacey et al., 1993), but their functional activities have not so far been described at central $GABA_B$ receptor sites. Here, in rat neocortical slices, we show that the thienyl analogs of baclofen, 5d, 5f and 5h, together with 4-amino-3-(thien-3-yl)-butanoic acid (5j) (Berthelot et al., 1991) are indeed also full agonists at central $GABA_B$ receptors, albeit less potent than baclofen itself.

2. Materials and methods

2.1. Rat neocortical slice preparations

Outbred male adult Sprague-Dawley rats (250–350 g) were decapitated, their brains rapidly removed and im-

* Corresponding author. Tel.: (61-8) 8303-5163; Fax: (61-8) 8303-3788; e-mail: jong@medicine.adelaide.edu.au

PII S0014-2999(97)00185-4

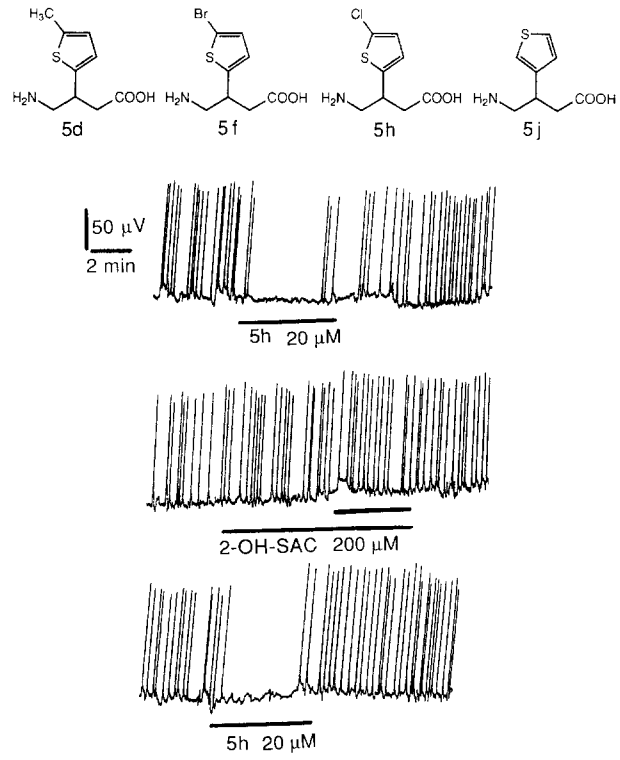

Fig. 1. Suppression of spontaneous discharges, in rat isolated neocortical slice preparations maintained in Mg^{2+}-free medium, by 4-amino-3-(5-chlorothien-2-yl)-butanoic acid (5h). The chemical structures of 4-amino-3-(5-methylthien-2-yl)-butanoic acid (5d), 4-amino-3-(5-bromothien-2-yl)-butanoic acid (5f), 4-amino-3-(5-chlorothien-2-yl)-butanoic acid (5h), and 4-amino-3-(thien-3-yl)-butanoic acid (5j) are shown. The suppression of spontaneous activity by 5h (20 μM) was reversibly antagonised by the $GABA_B$ receptor antagonist 2-hydroxysaclofen (2-OH-SAC; 200 μM). The control response to 5h was subsequently re-established upon washing out the antagonist ($n = 6$).

mersed for 15 min in ice-cold Krebs solution oxygenated with 95% O_2 and 5% CO_2. Cerebral cortical slices (400 μm thick) were prepared by cutting coronal sections using a vibraslice microtome (Campden Instruments, UK), and a radial wedge was cut from each side of the dorsal mid-line to yield slices of cingulate cortex and corpus callosum 1.5–2 mm wide. Using a superfusion method based on a grease-gap system (Ong et al., 1990), the neocortex was initially superfused with Mg^{2+}-containing Krebs medium at 28°C delivered by a peristaltic pump at 1 ml/min, and allowed to equilibrate for 30 min, followed by Mg^{2+}-free medium. The composition of the Krebs medium was as follows (mM): NaCl 118, KCl 2.1, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11, $MgSO_4$ 1.3, pH 7.4. For the Mg^{2+}-free medium, $MgSO_4$ was omitted. DC potentials between the cingulate cortex and corpus callosum were monitored by Ag/AgCl electrodes via agar/saline bridges with a high-input impedance DC amplifier, and responses displayed on a chart recorder.

After a period of equilibration for 60 min under Mg^{2+}-free conditions, the neocortical slices developed spontaneous paroxysmal discharges. Drugs added to the superfusing medium were subsequently applied to the cortical side of the tissues for 5–10 min, usually at 30 min intervals depending on the recovery of the responses to control level. In this arrangement, recording cortex against corpus callosum, depolarising agents such as KCl (5 mM) or *N*-methyl-D-aspartate (10 μM) when superfused over the cortical side, caused an upward deflection as did the spontaneous paroxysmal depolarisations in Mg^{2+}-free medium. Each experiment was repeated on 6 slices from 3 different animals. Agonists were applied to the cortical side of the tissues, and the antagonist was added together with each agonist. Each experiment was repeated on 6 slices from 3 different animals. Data were analysed by counting the number of spontaneous discharges over a 5 min period, in the absence and presence of a compound, and the frequency expressed as the number of discharges per minute. The effects of the different substances tested are given as % of control discharges. All results are expressed as mean value ± S.E.M. Statistical significance was determined by Student's *t*-test for unpaired samples (significance level $P < 0.05$).

2.2. Drugs

The thienyl analogs 4-amino-3-(5-chlorothien-2-yl)-butanoic acid (5h), 4-amino-3-(5-methylthien-2-yl)-butanoic acid (5d), 4-amino-3-(5-bromothien-2-yl)-butanoic acid (5f), and 4-amino-3-(thien-3-yl)-butanoic acid (5j) were synthesised as racemates by Dr. Berthelot and his colleagues at Lille; bicuculline methiodide was from RBI, and 2-hydroxysaclofen was a gift from Professor R.H. Prager (Flinders University, South Australia), whilst baclofen was a gift from Ciba-Geigy (Basel, Switzerland).

3. Results

In rat neocortical slices, repetitive paroxysmal discharges, with afterpotentials, usually developed within an hour of removing Mg^{2+} from the superfusing medium. The frequency of occurrence and the duration of the spontaneous activity varied considerably between different preparations, but activity was quantifiable by counting the number of discharges over a 5 min period, and expressed as number of responses per minute. Superfusion of the $GABA_B$ receptor agonist baclofen over the slice briefly depressed the amplitude, and subsequently abolished the discharges or reduced their frequency. This was prevented by the $GABA_B$ receptor antagonist 2-hydroxysaclofen (200 μM; data not shown). As shown in Table 1, baclofen (5 and 10 μM) dose-dependently reduced the frequency of spontaneous activity; this effect was seen as low as 2 μM, and at 10 μM, the discharges were almost completely blocked. The EC_{50} for baclofen in suppressing the discharges was 5 μM.

Table 1
Effects of baclofen and thienyl analogs on frequency of spontaneous discharges in rat neocortical slices

Drugs	Frequency of discharges (% of control)
Control	100
Bac (2 μM)	80 ± 5
5h (4 μM)	79 ± 8
5d (8 μM)	82 ± 4
5f (12 μM)	77 ± 6
Bac (5 μM)	51 ± 11
5h (10 μM)	54 ± 10
5d (20 μM)	48 ± 8
5f (30 μM)	44 ± 6
Bac (10 μM)	10 ± 5
5h (20 μM)	14 ± 7
5d (40 μM)	16 ± 6
5f (60 μM)	18 ± 8

Dose-related effects of baclofen and the different heterocyclic analogs, 4-amino-3-(5-methylthien-2-yl)-butanoic acid (5d), 4-amino-3-(5-bromothien-2-yl)-butanoic acid (5f), and 4-amino-3-(5-chlorothien-2-yl)-butanoic acid (5h), in suppressing spontaneous activity in the rat neocortex. Data are expressed as a percentage of the control value measured prior to any drug treatment, showing mean values ± S.E.M. All drug effects are significantly different from the control ($P < 0.05$; $n = 6$).

Similarly to baclofen, the thienyl analogs of baclofen 5d, 5f, 5h and 5j were full $GABA_B$ receptor agonists that reduced the frequency of spontaneous discharges. The $GABA_B$ receptor antagonist 2-hydroxysaclofen (200 μM) alone did not affect the spontaneous discharges but reversibly abolished the attenuation of spontaneous activity induced by these compounds. In experiments representative of six studies, baclofen ($EC_{50} = 5$ μM) was twice more potent than 5h ($EC_{50} = 11$ μM), but was four times, and five times more potent than 5d ($EC_{50} = 20$ μM), and 5f ($EC_{50} = 26$ μM), respectively, in abolishing the activity (Table 1). By contrast, the thien-3-yl analog 5j was 40 times weaker than baclofen (Figure not shown), being even weaker than the thien-2-yl analog (Ong et al., 1992b). The heterocyclic analog 5h (20 μM) significantly reduced the spontaneous activity, reversibly antagonised by 2-hydroxysaclofen (200 μM), with a recovery of the response to 5h following washout periods of 30 min (Fig. 1). Neither baclofen nor any of the thienyl analogs of baclofen showed any detectable desensitization when superfused onto the preparations, nor were they sensitive to the $GABA_A$ receptor antagonist bicuculline methiodide (10 μM).

4. Discussion

Using rat neocortical slices, the present series of racemic thienyl analogs of baclofen were all full agonists at central $GABA_B$ receptors, although less potent than (*R*,*S*)-baclofen, their relative potencies being baclofen > 5h > 5d > 5f ≫ 5j. This rank order is similar to that previously found in binding studies (Berthelot et al., 1991), and in the spinal cord (Lacey et al., 1993), as well as at peripheral $GABA_B$ receptors in the guinea-pig ileum (Ong et al., 1992a). Along with the corresponding furyl derivatives, these thienyl analogs of baclofen illustrate the importance of aromaticity at the β-substituent for $GABA_B$ receptor affinity. The present results thus provide important structure-action verification of the 'baclofen' pharmacophore at $GABA_B$ receptors (for details see Pirard et al., 1995).

The absolute configuration of (*R*)-baclofen is known, with the β-phenyl substituent perpendicular to the plane of the zig-zag, semi-extended GABA backbone (Chang et al., 1982; Kerr and Ong, 1995). In the present active analogs, the thienyl rings assume a similar conformation, evidently due to the mutual repulsion between the positivity of the basic functionality and that on the sulfur of the thienyl ring (Pirard et al., 1995). In the absence of information on the structure of $GABA_B$ receptors, it is not possible to assign a definite function to the hydrophobic 5-substituent, or its binding, in compounds 5h, 5d or 5f, but the most active analog was 5h. In this analog, the hetero ring has considerable aromaticity, and the 5-chloro-substituent evidently occupies the same space as does the 4-chloro on the phenyl ring of baclofen. The same is true of the next most active 5-methyl-substituted analog (5d), where the methyl can be considered a bioisostere of the 5-chloro-substituent in 5h. On the other hand, the 5-bromo-substituent in 5f is evidently too bulky for fully effective binding, iso-propyl being its equivalent bioisostere, with a reduction in agonist potency. The least active congener 5j is only a weak central agonist, where the positivity at the sulfur of the hetero ring is in an unfavourable position that markedly depresses binding. The corresponding 3-(thien-2-yl) analog (5c) (Berthelot et al., 1991), which lacks the essential 5-chloro or 5-methyl moiety of the more active analogs, is only a weak agonist, albeit more potent than 5j at central $GABA_B$ receptors (Ong et al., 1992a). However, the favourable 5-chloro substituent in 5h provides an agonist half as potent as baclofen, that may prove useful in functional studies.

Acknowledgements

We thank the Australian Research Council (ARC) for financial support. J.O. is an ARC Senior Research Fellow.

References

Berthelot, P., Vaccher, C., Flouquet, N., Luyckx, M., Brunet, C., Boulanger, T., Frippiat, J.P., Vercautere, D.P., Debaert, M., Evrard, G., Durant, F., 1991. Synthesis, pharmacology and X-ray studies of baclofen analogues. Eur. J. Med. Chem. 26, 395–402.

Bowery, N.G., Doble, A., Hill, D.R., Hudson, A.L., Shaw, J.S., Turnbull,

M.J., Warrington, R., 1981. Bicuculline-insensitive GABA receptors on peripheral autonomic nerve terminals. Eur. J. Pharmacol. 71, 53–70.

Carruthers, N.I., Spitler, J.M., Wong, S.C., Blythin, D.J., Chen, X., Shue, H.J., Mittelman, S., 1995. Synthesis and resolution of β-(aminomethyl)-4-chlorobenzeneethanesulfinic acid a potent $GABA_B$ receptor ligand. Biorg. Med. Chem. Lett. 5, 237–240.

Chang, C.H., Yang, D.S.C., Yoo, C.S., Wang, B.C., Pletcher, J., Sax, M., Terrence, C.F., 1982. Structure and absolute configuration of (*R*)-baclofen monochloride. Acta Cryst. B38, 2065–2067.

Froestl, W., Mickel, S.J., Hall, R.G., Von Sprecher, G., Strub, D., Baumann, P.A., Brugger, F., Gentsch, C., Jaekel, J., Olpe, H.R., Rihs, G., Vassout, I.A., Waldmeier, P.C., Bittiger, H., 1995. Phosphinic acid analogues of GABA 1. New potent and selective $GABA_B$ agonists. J. Med. Chem. 38, 3297–3312.

Kerr, D.I.B., Ong, J., 1995. $GABA_B$ receptors. Pharmacol. Ther. 67, 187–246.

Lacey, G., Berthelot, P., Vaccher, C., Flouquet, N., Vaccher, M.P., Debaert, M., Curtis, D.R., 1993. Thienyl-GABA derivatives as specific baclofen agonists in the rat and cat spinal cord in vivo. Neurosci. Lett. 159, 64–66.

Ong, J., Kerr, D.I.B., Johnston, G.A.R., 1990. Effects of potassium channel blockers on baclofen-induced suppression of paroxysmal discharges in rat neocortical slices. Neurosci. Lett. 112, 216–222.

Ong, J., Kerr, D.I.B., Berthelot, P., Vaccher, C., Flouquet, N., Debaert, M., 1992a. Actions of thienyl analogs of baclofen in the guinea-pig isolated ileum. Eur. J. Pharmacol. 218, 343–345.

Ong, J., Kerr, D.I.B., Berthelot, P., Vaccher, C., Flouquet, N., Debaert, M., 1992b. Differing actions of β-(2-thienyl)-GABA in central and peripheral preparations. Eur. J. Pharmacol. 221, 145–149.

Pirard, B., Paquet, B., Evrard, G., Berthelot, P., Vaccher, C., Ansard, M.H., Debaert, M., Durant, F., 1995. $GABA_B$ pharmacophoric pattern based on conformational analysis of 3-heteroaromatic baclofen analogues. Eur. J. Med. Chem. 30, 851–857.