Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1759–1761

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Simple Polar Deacetylated Caloporoside Derivative is a Positive Modulator of the $GABA_A$ Chloride Channel Complex in Cortical Mammalian Neurones

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Received 7 April 2000; accepted 6 June 2000

Abstract—Synthesis of octyl-*O*-β-D-mannopyranoside, a caloporoside analogue was achieved by the activation of 2,3,4,6-*tetra*-*O*-benzyl-1-*O*-1′,3′2′-dioxaphosphacyclohexane-α,β-D-mannopyranosyl-2-oxide with TMSOTf (Trimethyl silyl triflate) and subsequent debenzylation. At 100 μM the molecule significantly and reversibly increased the magnitude of $GABA_A$ currents evoked in cultured rat pyramidal neurones whilst concomitantly reducing the incidence of spontaneous synaptic activity. These results contradict earlier proposals that such molecules bind to the TBPS (*tert*-Butylbicyclophosphorothionate) site to block the chloride channel.

GABA is the most widespread inhibitory transmitter in the brain where it is present in nerve terminals at ca. one third of all CNS synapses.[1] The $GABA_A$ receptor is a ligand-gated chloride channel which is the target for a variety of commercially important depressant drugs including benzodiazepines, barbiturates, neurosteroids and a variety of volatile anaesthetic agents.[2,3] The clinically useful congeners allosterically enhance the magnitude and or duration of inhibitory synaptic currents to exert their sedative, hypnotic or anaesthetic effects. However, within each class of molecules certain congeners can produce antagonistic actions resulting in reduction of chloride currents, hyperexcitation in the CNS and convulsions in whole animals.[4–6] Flumazenil is an selective antagonist of the benzodiazepine site on the channel protein which competitively displaces both depressant and sedative congeners from their recognition site without exerting a physiological response.[7] Halogenated insecticides like the γ isomer of hexachlorocylohexane (lindane) block the GABA activated chloride channels expressed on arthropod muscle or neurones but the non insecticidal β analogue enhances chloride currents.[8] The bi-functional nature of this allosteric coupling means that it is difficult to interpret simple radioligand binding studies in isolation[6] (as this reveals only affinity for the complex but gives no indication of intrinsic efficacy to act as an agonist, antagonist or inverse-agonist). The natural product deacetyl-caloporiside has been reported to displace TBPS from its site in the lumen of the chloride channel which has been interpreted as evidence that it blocks the $GABA_A$ receptor complex.[9–11] Here, we have synthesised a very simple polar caloporoside analogue, octyl-*0*-β-D-mannopyranoside **3**, from 2,3,4,6-*tetra*-*O*-benzyl-1-*O*-1′,3′2′ -dioxaphosphacyclohexane-α,β-D-mannopyranosyl-2-oxide **1**[12] and subsequent debenzylation of **2**β with palladium hydroxide, (Scheme 1)[13] and characterised its functional effects on inhibitory ion channels.

Details of cell-culture and electrophysiological methods have been published in full.[14] Briefly, whole cell patch clamp recording was used with 70–80% series resistance compensation applied at the axopatch. Extracellular salines and the gluconate-based pipette solutions allow us to resolve inhibitory synaptic currents (IPSCs) from excitatory currents (EPSCs) and characterise spontaneous synaptic activity.[15] Briefly we applied 2s pulses of 10 μM GABA (every 60s) to rat cortical pyramidal cells maintained in culture for 14–28 days using a Y-tube (rapid application across entire cell). The octyl-*O*-β-D-mannopyranoside **3** was dissolved initially in dimethylsulphoxide (DMSO) and diluted 1:1000 into test salines. To control for solvent artefacts, 0.1% DMSO was added routinely to drug free salines and had no effect on any of the parameters reported here. The saccharide **3** was applied to the cell by superfusion between pulses and also added to the agonist solution to assess the modulatory response under equilibrium conditions. Data were analysed using CED and Graphpad software: all data are cited as mean ± standard error of the mean.

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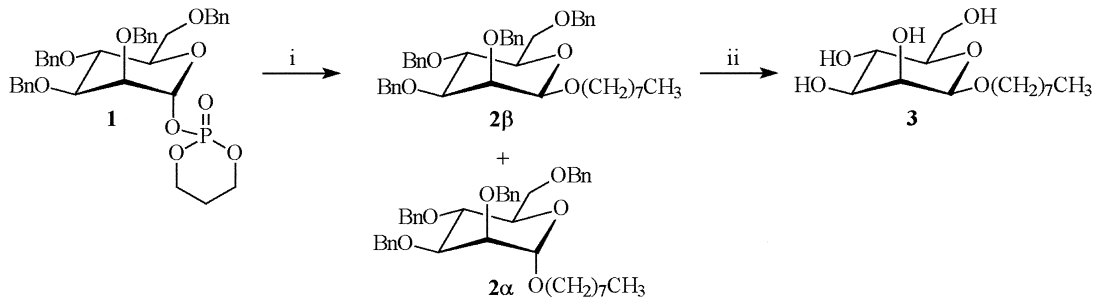
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00330-9



Scheme 1. Reagents and Conditions: (i) $HO(CH_2)_7CH_3$, TMSOTf (cat.), −78 °C, 0.5 h; (ii) Pd $(OH)_2$, MeOH, cyclohexene, 12 h.

100 μM **3** enhanced the response to GABA in all 10 cells examined (Fig. 1a). Onset of the response was complete within ca. 2–3 min and at equilibrium the currents increased to 131 ± 4.3% of the pretreatment responses (Fig. 1b). Under identical conditions, in previous studies, both the sleep modulator oleamide (at 20 μM) and the inhalational anaesthetic isoflurane (320 μM) enhanced GABA currents by a similar magnitude (<2-fold increase).[14,16] A two-tailed paired *t*-test revealed that the **3** effect was highly significant ($p = 0.0025$). Like the depressant drug and the sleep hormone above, 100 μM **3** also reversibly reduced the incidence of both spontaneous excitatory post-synaptic currents and inhibitory post-synaptic currents (Fig. 1c).

These results strongly suggest that even the simple amphiphilic alkylated mannose synthesised here has the capacity to mimic caloporoside in binding to the $GABA_A$ complex in mammalian brain membranes,[9] albeit with relatively low affinity. However, our data demonstrate that functionally the novel polar molecule is a positive modulator of the channel complex and exerts similar effects to those of commonly used depressant drugs and a putative sleep hormone.

Many chloride channel antagonists (like picrotoxinin and halogenated insecticides) are very apolar and block the channel with high affinity and reverse slowly if at all.[8] The saccharide **3** is a water-soluble ligand which can access its target site relatively quickly and reversibly. Future studies will address whether the molecule exerts an open-channel or use-dependent modulatory effect (i.e., whether the molecule accesses its recognition site via the channel lumen). Deacetyl-caloporoside (which we were unable to procure for these studies) displaces TBPS from its recognition site in the lumen of the chloride channel site but has not been examined in a functional assay to date. By inference the authors of studies on the natural product and its synthetic counterpart suggest that the molecules are "inhibitors" of the $GABA_A$ receptor ion channel.[9] The commercial importance of the $GABA_A$ receptor as a drug and insecticide target is well established. However, we still do not have ideal anxiolytic, sedative, or hypnotic drugs for chronic treatments. Our data highlights the importance of functional characterisation of channel-directed ligands to indicate the physiological relevance of pharmacophore modelling based solely on binding profiles. Caloporoside and octyl-*O*-β-D-mannoyranoside **3** may occupy separate binding sites on the ligand-gated ion channel complex but only further studies combining radiolabelled probes with functional studies (micro-electrodes or ion fluxing) can address this issue or resolve whether, like benzodiazepines, a single receptor can explain the actions of sedative and convulsant drugs. Such molecules, if they can bypass the blood-brain barrier, warrant further study as potential drugs for important pharmaceutical markets.[17]

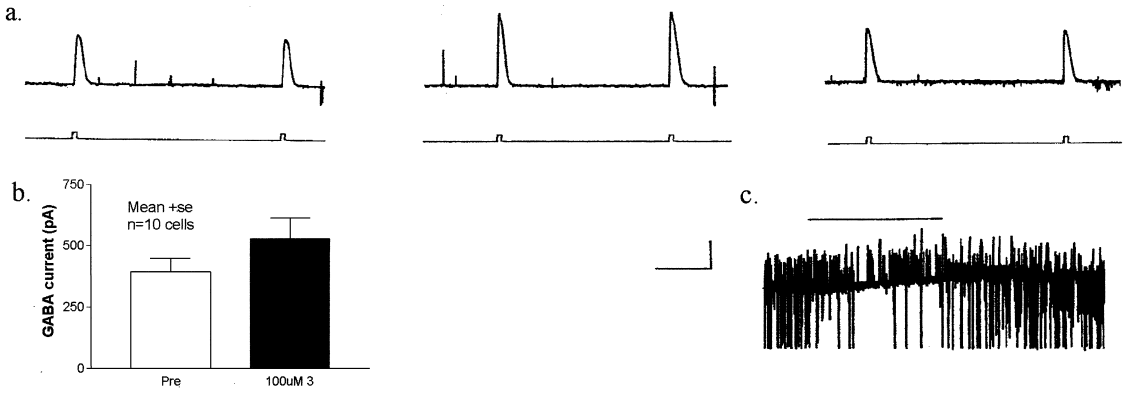


Figure 1. (a) Pairs of responses from the same cell to 10 μM GABA before (left), during (centre) and after (right) the administration of 100 μM **3**. Upper traces represent currents from the cell clamped at −45 mV; lower traces indicate timing and duration of GABA pulses. Scale bars: 400 pA, 24s; (b) Column graph depicting data from ten replicated experiments. The difference between the means was highly significant ($p = 0.0025$); (c) compressed recording from another cell showing typical effects of 100 μM **3** (horizontal bar) on spontaneous synaptic traffic. Note the reversible depression in the incidence of both IPSCs (upward deflections) and EPSCs (small downward defiections). Action currents (large downward deflections) represent action potential firing in the patch-clamped neurone were particularly sensitive in this cell. Scale bars: 400 pA, 2 min.

Acknowledgements

We (GL and PLC) thank The Welcome Trust for equipment support.

References and Notes

1. Bloom, F. E.; Iversen, L. L. *Nature* **1971**, *229*, 628.
2. Johnston, G. A. R. *Pharmacol. Ther.* **1996**, *69*, 173.
3. Belelli, D.; Pistis, M.; Peters, J. A.; Lambert, J. J. *TIPS* **1999**, *20*, 496.
4. Cottrell, G. A.; Lambert, J. J.; Peters, J. A. *Br. J. Pharmacol.* **1987**, *90*, 491.
5. Wakamori, M.; Ikemoto, Y.; Akaike, N. *J. Neurophysiol.* **1991**, *66*, 2014.
6. Maksay, G.; Molnar, P.; Simonyi, M. *Naunyn-Schmiedebergs Archives Of Pharmacology* **1996**, *353*, 306.
7. Hunkeler, W.; Mohler, M.; Pieri, L.; Dole, P.; Bonetti, E. P.; Cumin, R.; Schaffner, R.; Hafely, W. *Nature* **1981**, *290*, 514.
8. Lees, G.; Calder, J. *Pesticide Biochemistry and Physiology* **1996**, *55*, 40.
9. Tatsuta, K.; Yasuda, S. *Tetrahedron Letters* **1996**, *37*, 2453.
10. Crich, D.; Barba, G. R. *Tetrahedron Letters* **1998**, *39*, 9339.
11. Shan, R.; Anke, H.; Neilsen, M.; Sterner, O.; Witt, M. R. *Nat. Prod. Lett.* **1994**, *4*, 171.
12. Singh, G.; Vankayalapati, H. *Tetrahedron: Asymmetry* **2000**, *11*, 125.
13. Data for **3**: $[\alpha]_d^{22}$ +29.5 (c 2.2, $CHCl_3$); δ_H (270 MHz; $CDCl_3$) 0.84 (3H, t, *J* 6.59, 5.94), 1.25, (10H, bs), 1.52 (2H, s), 3.34 (1H, d, *J* 7.91), 3.46 (1H, d, *J* 7.26), 3.58 (1H, d, *J*, 6.60), 3.70 (1H, d, *J* 11.22), 3.83–3.89 (4H, m), 4.78 (1H, bs); δ_C, (67.8 MHz; $CDCl_3$) 14.03, 22.59, 26.05, 29.20, 29,37, 29.63, 31.80, 60.67, 65.93, 67.84, 70.99, 71.45, 72.23, 100.01; *m/z* (CI, NH_3) Found: $(M^+ + NH_4)$ 310.2222 $C_{14}H_{32}NO_6$ requires 310.2230.
14. Lees, G.; Edwards, M. D.; Hassoni, A. A.; Ganellin, C. R.; Galanakis, D. *Br. J. Pharmacol.* **1998**, *124*, 873.
15. Verdon, B.; Zheng, J.; Nicholson, R.; Ganellin, C.; Lees, G. *Br. J. Pharmacol.* **2000**, *129*, 283.
16. Lees, G.; Edwards, M. D. *Anesthesiology* **1998**, *88*, 206.
17. Costa, E.; Guidotti, E. *TIPS* **1996**, *17*, 192.