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Radioligand binding studies of caloporoside and novel congeners with contrasting effects upon [^{35}S] TBPS binding to the mammalian $GABA_A$ receptor

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Abstract

Caloporoside is a natural active fungal metabolite, which was isolated from *Caloporous dichrous* and was described to exhibit antibacterial, antifungal and phospholipase C inhibitory activity. We have previously reported evidence that related β-linked compounds, lactose and octyl-β-D-mannoside, bind and functionally modulate rodent $GABA_A$ receptors, respectively. We have characterized the binding pharmacology of synthetic caloporoside and two further congeners, 2-hydroxy-6-{[(16R)-(β-D-mannopyranosyloxy)heptadecyl]} benzoic acid and octyl-β-D-glucoside on $GABA_A$ receptors using a [^{35}S]-*t*-butylbicyclophosphoorothionate (TBPS) radioligand binding assay. Caloporoside and 2-hydroxy-6-{[(16R)-(β-D-mannopyranosyloxy)heptadecyl]} benzoic acid produced concentration-dependent complete inhibition of specific [^{35}S] TBPS binding with overall apparent IC_{50} values of 14.7 ± 0.1 and 14.2 ± 0.1 μM, respectively. In contrast, octyl-β-D-glucoside elicited a concentration-dependent stimulation of specific [^{35}S] TBPS binding ($E_{max} = 144 \pm 4\%$; $EC_{50} = 39.2 \pm 22.7$ nM). The level of stimulation was similar to that elicited by diazepam ($E_{max} = 147 \pm 6\%$; $EC_{50} = 0.8 \pm 0.1$ nM), and was occluded by GABA (0.3 μM). However, the three test compounds failed to elicit any significant effect (positive or negative) upon [^{3}H] flunitrazepam or [^{3}H] muscimol binding, indicating that they did not bind directly, or allosterically couple, to the benzodiazepine or agonist binding site of the $GABA_A$ receptor, respectively. The constituent monosaccharide, glucose, and both the closely related congeners octyl-β-D-glucoside or hexyl-β-D-glucoside have no significant effect upon [^{35}S] TBPS binding. These data, together, provide strong evidence that a β-glycosidic linkage and chain length are crucial for the positive modulation of [^{35}S] TBPS binding to the $GABA_A$ receptor by this novel chemical class.

Keywords: $GABA_A$ receptor; Allosteric modulator; TBPS; SAR; Channel site; Antagonist; β-Linkage; Sugar

1. Introduction

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in mammalian central nervous system. It is involved in a wide spectrum of physiological functions and behaviours, through binding to the ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors, respectively. Actions of several important classes of clinically used drugs, such as benzodiazepines, barbiturates and anaesthetics, are at least partly mediated by allosteric interactions at the $GABA_A$ receptors [1,2].

The great molecular diversity of the multisubunit hetero-oligomeric $GABA_A$ receptors provides opportunities to develop novel drugs, e.g. for anxiety, sleep disorders, alcoholism and epilepsy, by establishing the relevant molecular targets for receptor subtype-specific action [3–5].

The high-affinity binding displayed by cage convulsants, such as TBPS, have proven to be useful in the development of radioligands for $GABA_A$ receptors and for their subsequent in vitro biochemical and pharmacological char-

Abbreviations: GABA, γ-aminobutyric acid; TBPS, *t*-butylbicyclophosphoorothionate; SAR, structure activity relationship; TBOB, *t*-butylbicycloorthobenzoate; DMSO, dimethylsulphoxide

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acterization [5]. These studies have revealed that $GABA_A$ receptors have multiple allosteric binding sites for drugs which, when occupied, modulate (positively or negatively) the inhibitory actions of GABA [1,2,5,6].

Caloporoside is a natural active fungal metabolite, which was isolated several years ago from fermentation of *Caloporous dichrous*, and was originally described to exhibit weak antibacterial and antifungal activity, as well as phospholipase C inhibitory activity [7]. In the same year, two related secondary metabolites were isolated from the same fungus species, and were reported, in a preliminary study, to act as inhibitors of [^{35}S] TBPS binding to the $GABA_A$/benzodiazepine chloride channel receptor complex in vitro [8]. Synthesis and biological evaluation of the caloporoside analogue, deacetylated caloporoside, has been reported [9,10]. The compound appeared to display modest binding affinity for the $GABA_A$ receptor channel (cited $IC_{50} = 40–60$ μM) [9,10]. Detailed pharmacological analyses were lacking in these reports. The chemical structure of caloporoside was elucidated by combination of chemical and spectroscopic methods [7,8,11]. Caloporoside consists of salicylic acid and a β-D-mannopyranosyl-D-mannonic acid moiety which are linked by an alkyl chain; the sugar part carries two acetyl residues at the 2- and 2′-position. Analogues of this compound have been described [11] which differ from the natural product in the aldohexose and the aldonic acid part. For example, the sugar moiety may be D-mannopyrannosyl-D-mannoic acid, which can be unsubstituted or substituted [11].

Successful chemical synthesis of the physiologically active fungal metabolite caloporoside has been described by our group [12,13]. The published procedure permits the synthesis of caloporoside and other closely related analogues, which may prove to be promising compounds for further biological evaluation. The other interesting issue relates to the sugar moiety of caloporoside, which is characterized by the highly unusual β- (1 → 5) linkage of a D-mannopyranoside unit to a D-mannonate ester. The stereoselective chemical synthesis of the β-mannopyranosidic linkage is not a trivial issue in carbohydrate chemistry, however, practical syntheses of β-mannopyranosides have been described [14,15]. Recently, a new strategy for the synthesis of mannopyranoside was reported [16,17].

Our laboratory showed that a simple polar deacetylated caloporoside derivative is a positive functional modulator of the $GABA_A$ chloride channel. Octyl-β-D mannopyranoside (100 μM) significantly and reversibly increased the magnitude of $GABA_A$ currents evoked in the cultured rat cortical pyramidal neurons [18]. A subsequent study demonstrated that a simple β-linked disaccharide, lactose, but not the α-linked disaccharides maltose or sucrose, can bind the $GABA_A$ receptor channel, detected by positive modulation of [^{3}H] TBOB binding to the rodent $GABA_A$ receptor [6].

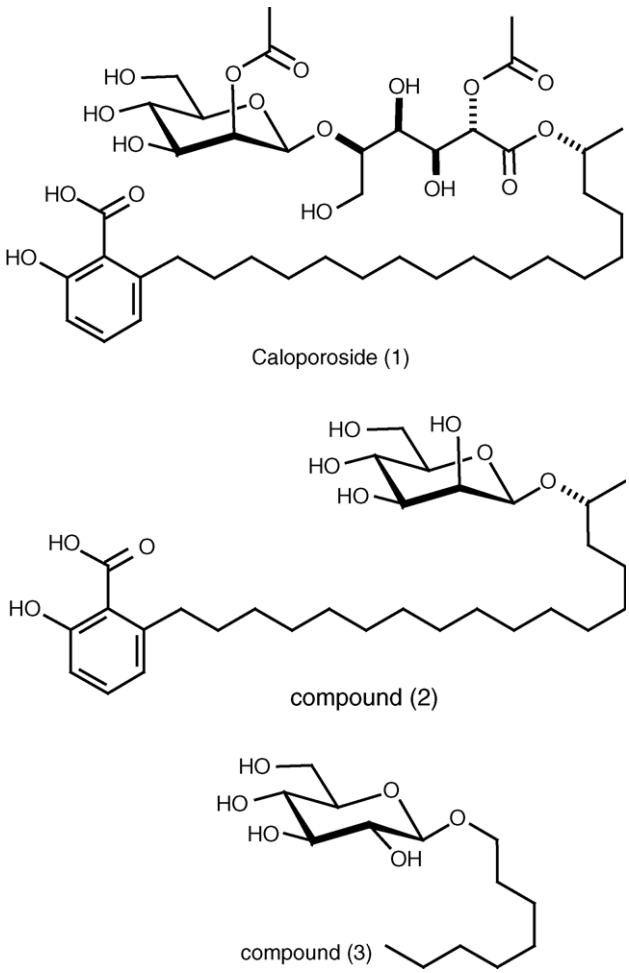

Fig. 1. Chemical structures of novel $GABA_A$ receptor compounds. Compound **1**, caloporoside; compound **2**, 2-hydroxy-6-{[(16R)-(β-D-mannopyranosyloxy)heptadecyl]} benzoic acid (HMHB); compound **3**, octyl-β-D-glucoside.

In this present report, we extend further the pharmacological binding profile of this new class of $GABA_A$ receptor ligand, using a radioligand binding approach with the high specific activity channel radioligand [^{35}S] TBPS. Three compounds, with the chemical structures shown in Fig. 1, were studied in the first instance: the synthetic parent molecule Caloporoside, 2-hydroxy-6-{[(16R)-(β-D-mannopyranosyloxy)heptadecyl]} benzoic acid (HMHB), which lacks the mannonic acid ester segment (compound **2**), and octyl-β-D-glucoside (compound **3**). We provide new evidence that synthetic caloporoside is a low affinity $GABA_A$ receptor ligand and in contrast, that the small polar congener, octyl-β-D-glucoside is a high-affinity positive modulator of [^{35}S] TBPS binding. Furthermore, we report that the modulatory activity of octyl-β-D-glucoside is dependent upon both the glycosidic linkage and length of the alkyl side chain.

A preliminary account of this work was reported recently in abstract form at the BPS conference in the University of Bath [19].

2. Materials and experimental procedures

2.1. Materials

[^{3}H] flunitrazepam, specific activity (91.0 Ci/mmol) was obtained from Amersham Biotech (Amersham), UK. [^{35}S]-*t*-butylbicyclophosphorothionate (TBPS), specific activity (80 Ci/mmol) from Perkin-Elmer Life Science, USA. [^{3}H] MK-801, specific activity (25 Ci/mmol) was obtained from ARC (USA). [^{3}H] muscimol, specific activity (36.5 Ci/mmol) was obtained from ARC (USA). Picrotoxinin, diazepam, γ-aminobutyric acid (GABA), octyl-α-D-glucoside, hexyl-β-D-glucoside, glutamate and ketamine were all obtained from Sigma Pharmaceuticals (Poole, UK). The three test compounds were synthesised in house, dissolved at 10^{-1} M in DMSO, and serial dilutions made with respective assay buffer. GABA stocks (10^{-2} M) were made in assay buffer. Diazepam stocks (10^{-2} M) were prepared in absolute ethanol. Picrotoxinin stocks (10^{-2} M) were prepared in DMSO. Ketamine stocks (10^{-2} M) were prepared in assay buffer. No effect of solvents on radioligand binding assays was seen at concentrations below 0.1% (v/v) DMSO or 0.1% (v/v) ethanol (data not shown).

2.2. Methods

A series of dose-response competition binding experiments were performed using [^{35}S] TBPS, [^{3}H] muscimol, [^{3}H] flunitrazepam and [^{3}H] MK-801 using well-washed adult rat forebrain membranes.

2.3. Tissue preparation

Adult male rats (200–300 g), Wistar strain, were maintained under a 12 h light, 12 h dark cycle at temperature of 23 °C and 65% humidity, with water and standard laboratory food available ad libidum. Animal treatment and husbandry were in accordance with approved use of animals in scientific procedures regulated by the Animals (Scientific Procedures) Act 1986, UK. The animals were killed humanely using a Schedule 1 procedure. The brains were rapidly removed, and the required tissue (forebrain) dissected immediately and kept cool on ice. The tissue was then homogenized in ice-cold homogenisation buffer (50 mM Tris–HCl, pH 7.4, containing 5 mM EDTA and 5 mM EGTA and 320 mM sucrose) using a dounce glass/glass homogenizer. The homogenate was centrifuged at 1000 × *g*, 4 °C for 10 min, the supernatant was stored in ice, and the pellets was re-homogenized in ice-cold buffer again, re-centrifuged at 1000 × *g*, 4 °C for 10 min. The supernatant from the first and second centrifugation steps were pooled together and centrifuged at 12,000 × *g*, 4 °C for 30 min. The supernatant was discarded and the pellet resuspended in 50 mM Tris containing 5 mM EDTA and 5 mM EGTA (5 ml/g of original tissue), and frozen at −20 °C.

2.4. Freeze-thaw protocol for the preparation of well-washed rat membranes

The $GABA_A$ receptor binding assays were performed with well-washed rat membranes prepared by a five-step freeze-thaw protocol [6]. The final aliquots (1 ml) were then frozen and stored at −20 °C.

2.5. Determination of protein concentration

The protein concentration was determined using the Lowry assay protocol [20] using Bovine serum albumin as the standard protein.

2.6. Radioligand binding assays

2.6.1. [^{35}S]-t-butylbicyclophosphorothionate (TBPS) binding assay

[^{35}S] TBPS binding was performed essentially as described in [21]. Briefly, well-washed rat membranes prepared by a five-step freeze-thaw protocol were, on the day of experiment, centrifuged and the supernatant was discarded. The pellets were resuspended in fresh 50 mM Tris buffer containing 0.2 M NaCl, pH 7.4, to yield a final protein concentration in the assay of 1 mg/ml. An amount of 100 μg membrane protein was incubated with [^{35}S] TBPS (approximately 20 nM) for 90 min at 25 °C with a range of test concentrations (10^{-11} to 10^{-4} M). This was sufficient incubation time to achieve equilibrium (data not shown). Non-specific binding was defined in the presence of 100 μM picrotoxinin.

2.6.2. [^{3}H] muscimol binding assay

[^{3}H] muscimol binding assays were performed as previously described in [22]. Briefly, well-washed rat forebrain membranes prepared by five-step freeze-thaw protocol were thawed, centrifuged and the supernatant was discarded. The pellets were resuspended again in fresh 50 mM Tris buffer pH 7.4 to yield a final protein concentration in the assay of 1 mg/ml. An amount of 100 μg membrane protein was incubated with [^{3}H] muscimol (approximately 10 nM) for 1 h at 4 °C with a range of test concentrations (10^{-11} to 10^{-4} M). Non-specific binding was defined in the presence of 100 μM GABA.

2.6.3. [^{3}H] flunitrazepam binding assay

[^{3}H] flunitrazepam binding assays were performed as previously described [6,23]. Briefly, well-washed rat forebrain membranes prepared by five-step freeze-thaw protocol were thawed, centrifuged and the supernatant was discarded. The pellets were resuspended again in fresh 50 mM Tris buffer containing 5 mM EDTA and 5 mM EGTA to yield a final protein concentration in the assay of 1 mg/ml. An amount of 100 μg membrane protein was incubated with [^{3}H] flunitrazepam (approximately 1 nM) for 1 h at 4 °C with a range of test concentrations (10^{-11} to

10^{-4} M). Non-specific binding was defined in the presence of 100 μM diazepam.

2.6.4. [^{3}H] MK-801 binding assay

[^{3}H] MK-801 binding assays were performed as previously described [24]. Briefly, well-washed rat forebrain membranes prepared by five-step freeze-thaw protocol were thawed, centrifuged and the supernatant was discarded. The pellets were resuspended again in fresh 25 mM sodium phosphate buffer pH 7.4, to yield a final protein concentration in the assay of 1 mg/ml. An amount of 100 μg membrane protein was incubated with [^{3}H] MK-801 (approximately 1 nM) and 10 μM glutamate for 2 h at 22 °C with a range of test concentrations (10^{-11} to 10^{-4} M). Non-specific binding was defined in the presence of 10 mM ketamine.

All four binding assays were terminated by rapid filtration through Whatman GF/B filters pre-soaked in phosphate buffer, which were washed (3 ml × 3 ml) using ice-cold 10 mM sodium phosphate buffer (pH 7.4), using a Brandel cell harvester. Filters were transferred into scintillation vials, liquid scintillation fluid added and incubated for 16–24 h at room temperature. The bound radioactivity was quantified using Beckman LS 500 CE scintillation spectrophotometer with a counting time of 4 min per vial.

2.7. Data analysis

Results from the radioligand binding assays were analysed using non-linear least squares regression (GraphPad Prism 4 Software). Curves were best fitted to a one- or two-site binding model as described in [6]. The EC_{50} and IC_{50} values are the concentrations for half-maximal enhancement and displacement, respectively. Data were analysed using a Student's unpaired *t*-test, with levels of significance set at $p < 0.05$.

3. Results

3.1. The effect of the test compounds on [^{35}S] TBPS binding to the $GABA_A$ receptor

Two control compounds were tested in order to validate the assay and membrane preparation. Picrotoxinin displayed a steep monophasic inhibition of [^{35}S] TBPS binding with Hill slope close to unity (nH = 1.28 ± 0.22; apparent IC_{50} = 0.33 ± 0.12 μM). In contrast, diazepam stimulated [^{35}S] TBPS binding with a mean E_{max} = 147 ± 6%; apparent EC_{50} = 0.80 ± 0.43 nM (mean ± S.D. for at least three individual experiments, Fig. 3B).

Caloporoside completely displaced specific [^{35}S] TBPS binding to well-washed membranes in a concentration-dependant manner. Data were best fit to a one-site binding model, with a pseudo Hill coefficient close to unity, yielding a mean apparent IC_{50} = 14.7 ± 0.11 μM. HMHB also completely displaced specific [^{35}S] TBPS binding to well-washed membranes in a concentration dependant manner. Data were best fit to a one-site binding model, yielding a mean apparent IC_{50} = 14.2 ± 0.1 μM (Fig. 2A and B). In contrast, octyl-β-D-glucoside stimulated [^{35}S] TBPS binding in a similar fashion to diazepam, yielding a mean E_{max} = 144 ± 4% and apparent EC_{50} = 39.2 ± 22.7 nM, respectively (Fig. 2C).

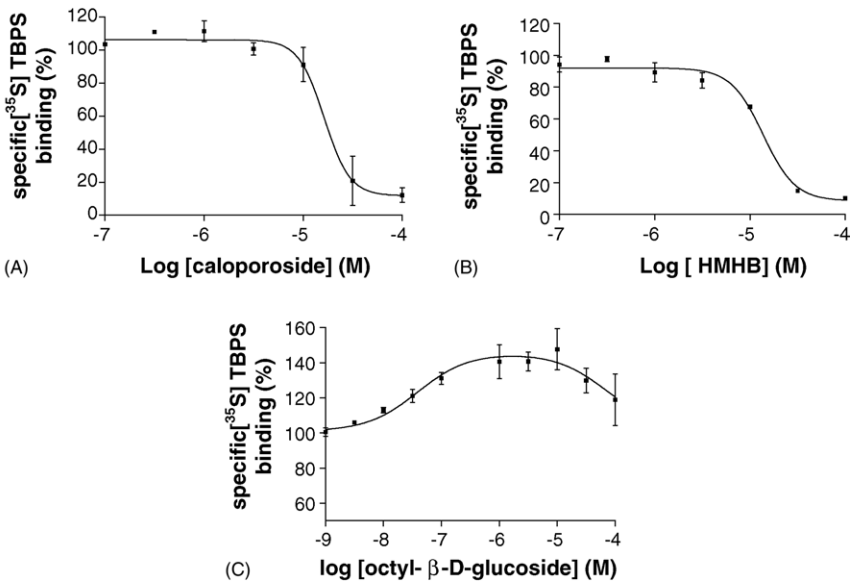

Fig. 2. Effect of caloporoside and congeners upon [^{35}S] TBPS binding to rat forebrain membranes. Effects of the compounds (A, caloporoside; B HMHB; C, octyl-β-D-glucoside) on specific [^{35}S] TBPS binding to well-washed adult rat forebrain membranes. Results are expressed as percentages (mean ± S.D. for three to six independent experiments) of control specific [^{35}S] TBPS binding in the absence of test compounds.

3.2. Sensitivity to GABA

In order to confirm that the stimulatory response was GABA-sensitive, 0.3 μM GABA was applied to the well-washed membranes. The presence of GABA partially reduced the overall [^{35}S] TBPS binding (by approximately 20%), and completely abolished the stimulation of [^{35}S] TBPS binding by both diazepam and octyl-β-D-glucoside (Fig. 3A and B). Octyl-β-D-glucoside failed to have any inhibitory or stimulatory effects in the presence of GABA.

3.3. The effect of octyl-β-D-glucoside on the agonist-binding site of the $GABA_A$ receptor labelled by [^{3}H] muscimol was investigated

In order to assess whether octyl-β-D-glucoside directly binds, or allosterically modulates muscimol binding to the agonist binding site, a range of concentrations of octyl-β-D-glucoside was tested upon [^{3}H] muscimol binding to a well-washed rat forebrain preparation. Specific binding was defined using 100 μM GABA. No significant effect (positive or negative) was detected across the full range of concentrations of octyl-β-D-glucoside in at least three independent experiments.

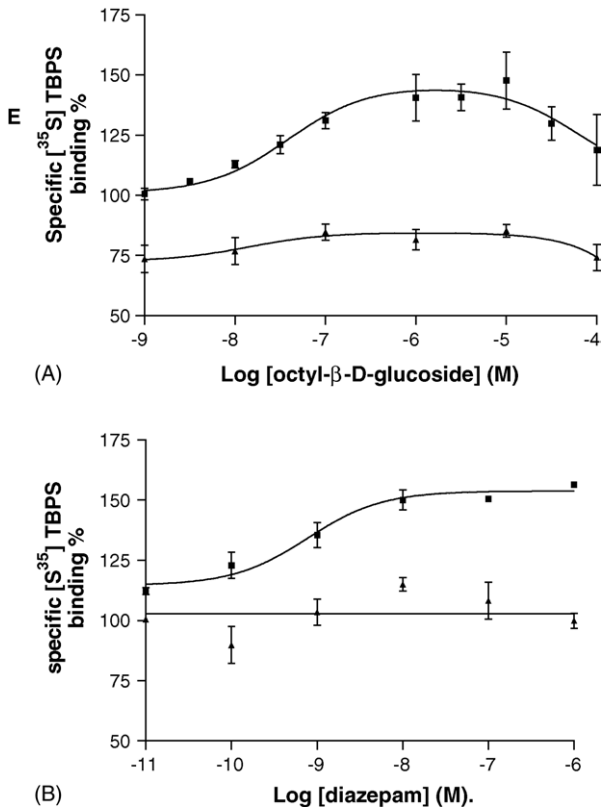

Fig. 3. Effect of GABA upon diazepam and octyl-β-D-glucoside modulation of [^{35}S] TBPS binding to rat forebrain membranes. Effect of diazepam or octyl-β-D-glucoside on specific [^{35}S] TBPS binding to well-washed adult rat forebrain membranes, in the absence (■) and presence of GABA (0.03 μM) (▼). Results are expressed as mean percentage values ± S.D. for three independent experiments.

3.4. The effect of the three compounds on the benzodiazepine-binding site of the $GABA_A$ receptor labelled by [^{3}H] flunitrazepam was investigated

In order to investigate whether caloporoside and the congeners are binding to the benzodiazepine site itself, a [^{3}H] flunitrazepam binding assay was used. Specific [^{3}H] flunitrazepam binding was defined using diazepam (100 μM) and represented >90% of the total binding (not shown). A control experiment of [^{3}H] flunitrazepam binding to rat forebrain, in the presence of different concentrations of GABA, was carried out to validate the assay. GABA significantly enhanced specific binding of [^{3}H] flunitrazepam to rat well washed forebrain in a concentration-dependent manner, yielding a mean $E_{max} = 153 \pm 4\%$ and apparent $EC_{50} = 31 \pm 20$ nM ($n = 3$ independent experiments).

In contrast, no significant effect (positive or negative) was observed with caloporoside, HMHB or octyl-β-D-glucoside upon [^{3}H] flunitrazepam binding in at least five independent experiments. This suggests a lack of interaction (either directly or allosterically) of these three compounds with the benzodiazepine site of the $GABA_A$ receptor. A small reduction in binding observed at a test concentration of 100 μM for the three compounds was due to the presence of 0.1% DMSO (solvent effect).

3.5. Influence of the side chain carbon length and stereochemistry

The core monosaccharide of compound **3**, i.e. glucose, was tested and found to have no significant effect upon [^{35}S] TBPS binding up to concentration of 100 μM (Table 1). Furthermore, neither octyl-α-D-glucoside nor hexyl-β-D-glucoside elicited significant effects upon [^{35}S] TBPS binding up to concentration of 100 μM (Table 1).

3.6. Does lactose bind to the same site as octyl-β-D-glucoside?

Previously, we showed that lactose potentiated [^{3}H] TBOB binding to the channel site of the $GABA_A$ receptor,

Table 1
Pharmacological effect of a range of related compounds upon [^{35}S] TBPS binding to adult rat forebrain membranes

Compound	Effect (10^{-10} to 10^{-4} M)
Glucose	NE
Hexyl-D-β-glucoside	NE
Octyl-D-β-glucoside	$EC_{50} = 39 \pm 23$ nM, $E_{max} = 144 \pm 4\%$
Octyl-D-α-glucoside	NE
Lactose (β-linked disaccharide)	NE
Octyl-D-β-glucoside + lactose (10^{-5} M)	NE

A series of related compounds were assayed for any potential effects upon [^{35}S] TBPS binding to well-washed adult rat forebrain membranes, over concentration range of 10^{-10} to 10^{-4} M ($n = 3$–6 separate experiments). NE, no significant effect detected (positive or negative) ($p > 0.5$).

with a maximal effect observed at 10 μM. In contrast, interestingly, lactose has no effect upon [^{35}S] TBPS binding up to 100 μM. However, we showed that lactose (10 μM) completely occluded the potentiation by octyl-α-D-glucoside of [^{35}S] TBPS binding. Octyl-β-D-glucoside failed to have any inhibitory or stimulatory effects in the presence of lactose (Table 1).

3.7. Selectivity of action of octyl-β-D-glucoside upon $GABA_A$ receptors

Octyl-β-D-glucoside had no effect (positive or negative) upon [^{3}H] MK-801 binding up to a 100 μM.

4. Discussion

The effects of caloporoside and two smaller congeners were assayed using a [^{35}S] TBPS binding assay on adult rat forebrain membranes. These data suggest that caloporoside and HMHB are low affinity $GABA_A$ receptor channel ligands, while, in contrast, octyl-β-D-glucoside is a relatively high affinity positive $GABA_A$ receptor channel modulator. The positive modulatory effect of octyl-β-D-glucoside was occluded in the presence of GABA, in a similar fashion to benzodiazepines, indicating that the modulatory action of octyl-glucoside is related to the conformational state of the chloride channel [25]. GABA sensitivity is shared by a number of other $GABA_A$ receptor modulators, as well as benzodiazepines, including loreclezole, propofol and lactose [6,25,26]. The lack of inhibitory action of octyl-β-D-glucoside at high concentrations is a property shared by diazepam, but not loreclezole or propofol. This property has been previously attributed to the lack of ability of diazepam to activate $GABA_A$ receptor channel in the absence of GABA [26].

The lack of effect of octyl-β-D-glucoside upon [^{3}H] muscimol binding demonstrated that octyl-β-D-glucoside does not directly bind to the agonist binding site. Based on shared properties of octyl-β-D-glucoside and diazepam in modulating [^{35}S] TBPS, we also directly investigated the effect of octyl-β-D-glucoside upon [^{3}H] flunitrazepam binding, using well-washed membranes. Neither caloporoside, HMHB nor octyl-β-D-glucoside displayed any significant (positive or negative) effect upon [^{3}H] flunitrazepam binding, which strongly suggested a lack of allosteric or competitive linkage with the benzodiazepine site. This property is in marked contrast to other ligands tested, such as GABA and diazepam, respectively. GABA positively modulates and diazepam competitively inhibited [^{3}H] flunitrazepam binding, consistent with previous studies. These data confirm that the novel compound class binds to a unique site on the $GABA_A$ receptor.

It should be noted that octyl-β-D-glucoside has been previously used as a detergent for the solubilisation of $GABA_A$ receptors, but at high mM concentrations (e.g. [27]). However, the propensity of octyl-β-D-glucoside to bind to membranes indicates that it may bind within the membrane spanning channel domain of the $GABA_A$ receptor. The lack of effect of octyl-β-D-glucoside upon channel binding of [^{3}H] MK-801 to another common ligand gated channel, namely the NMDA glutamate receptor suggests that octyl-β-D-glucoside does not bind non-selectively, and indiscriminately modulate all ligand-gated channels in the membrane.

Interestingly, the monosaccharide present in compound **3**, glucose had no significant effect upon [^{35}S] TBPS indicating that the presence of the extended side chain was absolutely necessary for $GABA_A$ receptor modulation. In order to investigate whether the nature of the glycosidic linkage is important, we compared, in parallel, the effects of octyl-α-D-glucoside, hexyl-β-D-glucoside and octyl-β-D-glucoside over the same concentration range. In contrast to octyl-β-D-glucoside, neither octyl-α-D-glucoside nor hexyl-β-D-glucoside significantly affected [^{35}S] TBPS binding. This strongly indicated that both the β-linkage and an alkyl side-chain in excess of 6-C in length, was crucial for the positive modulation of [^{35}S] TBPS binding. These results extend upon our previous observations with β- and α-linked disaccharides, which showed that β-glycosidic linkage yielded higher affinity $GABA_A$ receptor binding than α-glycosidic linkage [6].

Interestingly, lactose had no affect upon [^{35}S] TBPS which is in contrast to its affect upon [^{3}H] TBOB binding [6]. The differences in salt concentration in the two assays may explain this difference. Furthermore, the expanded structure of TBOB in comparison to TBPS may account for the differential allosteric influence of lactose and warrants further study. However, lactose (10 μM) completely blocked the positive modulation of [^{35}S] TBPS, which provides evidence for a shared binding site between these two β-glycosidic linked ligands.

In conclusion, this study has delineated clear differences in the pharmacological binding properties of the large natural product caloporoside and the small polar congener, octyl-β-D-glucoside. The findings reported in this study also provides evidence, firstly that octyl-β-D-glucoside binding is independent of the benzodiazepine and agonist binding sites, secondly, that the side chain is absolutely required for activity, and thirdly that glycosidic linkage and side chain length are important determinants of the modulatory activity. This present study has provided a clearer picture of the SAR of this novel class of $GABA_A$ receptor modulator, which warrants further elucidation using $GABA_A$ receptor electrophysiological and behavioural studies [18,28].

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