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Increased vigabatrin entry into the brain by polysorbate 80 and sodium caprate

D. Dimitrijevic, P. S. Whitton, M. Domin, K. Welham and A. T. Florence

Abstract

The effects of a non-ionic surfactant, polysorbate 80, and the sodium salt of the saturated fatty acid, sodium caprate (C10), as potential brain absorption enhancers for vigabatrin were studied. Vigabatrin is an enzyme-activated irreversible inhibitor of γ-aminobutyric acid (GABA) transaminase that increases brain and cerebrospinal GABA concentrations in animals and man. Before intravenous administration, a range of concentrations of the surfactants were tested using erythrocyte lysis or the red blood cell lysis test to establish the non-toxic concentration range. Vigabatrin was dissolved in 0.1% polysorbate 80 and 0.1% sodium caprate and administered intravenously in doses of 4 mL kg^{-1} to male Wistar rats (230–250 g; n = 3). Rats were killed 2 h after drug and surfactant administration and the brains were immediately removed and homogenized in 0.4 M perchloric acid. Selected ion monitoring electrospray mass spectrometry was used to determine the concentration of vigabatrin and GABA directly from the perchloric acid extract of the rat brain. This method was developed to increase the speed and efficiency of the analysis by removing the need for complex extraction and derivatization procedures while retaining the specificity of the mass spectrometer as a detector. The stability of both vigabatrin and GABA in perchloric acid was established by monitoring their pseudo molecular ions in standard solutions at timed intervals over 24 h. Although the detection level for vigabatrin and GABA was at least 50 pg, only GABA was detected in rat brain. Vigabatrin caused a small increase in whole brain GABA. However, GABA levels were higher in the samples with vigabatrin + enhancer than in the samples where vigabatrin alone was administered. One-way analysis of variance indicated a significant effect of the surfactants on GABA levels ($F(5,17) = 11.86$, $P < 0.01$) and vigabatrin absorption was presumed. The rectal temperature of the rats is lowered by the presence of vigabatrin in the brain. Vigabatrin alone decreased rectal temperature by 6%. When given with either polysorbate 80 or sodium caprate, the extent of temperature lowering was significantly greater ($P < 0.001$). There was no significant difference after 2 h between polysorbate 80 + vigabatrin, and sodium caprate + vigabatrin.

Centre for Drug Delivery Research, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK

D. Dimitrijevic, A. T. Florence

Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK

P. S. Whitton

Department of Pharmaceutical and Biological Chemistry, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK

M. Domin, K. Welham
Correspondence: A. T. Florence, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK. E-Mail: a.t.florence@ulsop.ac.uk
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Introduction

Surface active agents have been widely investigated as absorption enhancers for large or poorly absorbed hydrophilic molecules (Gibaldi & Feldman 1970; Florence 1981; Attwood & Florence 1983). Surface-active absorption enhancers increase the permeability of cell membranes in a concentration-dependent manner. It has generally been accepted that surfactants increase drug permeability by transcellular pathways, although some recent studies show that several absorption enhancers, such as sodium dodecyl sulfate, sodium caprate and long-chain acylcarnitines,

increase permeability through paracellular pathways (Hochman & Artursson 1994). Sodium caprate is an absorption enhancer used in rectal drug products in several countries including Japan and Sweden (Hochman & Artursson 1994). Polysorbate 80 is widely used in food stuffs and pharmaceuticals.

The aim of this study was to increase the absorption of vigabatrin into the brain. Vigabatrin (γ-vinyl GABA) is a relatively novel anticonvulsant drug effective in human and animal models of epilepsy. It acts by raising the synaptic concentration of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the CNS (Grant & Heel 1991; Halonen et al 1991; Mumford & Cannon 1994). Unlike many anti-epileptics the target for vigabatrin is located intracellulary; specifically vigabatrin acts as a suicide inhibitor of GABA-transaminase, thus preventing the breakdown of GABA. It has been shown to produce dose-dependent increases in cerebrospinal fluid GABA concentrations, and a decrease in GABA-transaminase activity (Connelly 1993). One major problem with vigabatrin is that it is a highly hydrophilic molecule with very poor penetration across the blood–brain barrier. As a result, although highly potent in-vitro, high doses of the drug (2–3 g daily in man) must be given to achieve an anticonvulsant/neuroprotective effect (Mattson et al 1995).

We studied the effect of a non-ionic surfactant, polysorbate 80, and the sodium salt of the saturated fatty acid, sodium caprate (C10), as potential absorption enhancers for vigabatrin. The effect of polysorbate 80 and sodium caprate on rat red blood cell stability was part of an initial study to determine non-toxic concentrations before intravenous administration.

GABA levels in plasma have been determined by HPLC, gas chromatography, and by gas chromatography interfaced to mass spectrometry (GC-MS) (Cann-Moisan et al 1990; Norman et al 1985; Schaaf 1985). Vigabatrin levels have been quantified in plasma by HPLC and GC-MS (Haegele et al 1983; Loscher et al 1993). GABA has also been determined in human cerebrospinal fluid or rat brain by mass fragmentography (Huizinga et al 1978; Nakamura et al 1987). In this study we used selected ion monitoring electrospray mass spectrometry to estimate vigabatrin and GABA levels directly from perchloric acid extracts of rat brain. The stability of both vigabatrin and GABA in perchloric acid was established by monitoring their pseudo molecular ions in standard solutions at timed intervals over 24 h. The rectal temperature of the rats, which is lowered by vigabatrin (Schechter & Tranier 1977), was monitored after drug administration.

Materials and Methods

Materials

Polysorbate 80, sodium caprate (C10), dextran, (MW 72000 Da), and sodium chloride were obtained from Sigma Chemicals, UK, and used as received. HPLC grade acetonitrile was from Fisher, UK, and perchloric acid was from BDH, UK. Vigabatrin (MW 129.16) was a gift from P. S. Whitton.

Effect of polysorbate 80 and capric acid on red blood cell stability (red blood cell lysis assay)

Polysorbate 80 and sodium caprate over the concentration range 0.005, 0.01, 0.05, 0.1, 0.5 and 1% were incubated with rat erythrocytes for 5 min, 1 h and 24 h at 37°C. The percentage of haemoglobin released with time was determined by spectrophotometric assay (ELISA microtiter plate reader) (Duncan et al 1994). The percentage haemoglobin released was calculated by reference to a control sample containing 1% Triton X-100 (100% haemoglobin release). Dextran was used as a negative control.

Mass spectrometry

Both vigabatrin and GABA were assayed directly in the 0.4 M perchloric acid extract of the rat brain by mass spectrometry. A Finnegan Navigator mass spectrometer interfaced to a Waters 2690 pump unit and autosampler was used. Electrospray ionization in the positive ion mode was used to generate pseudo molecular ions (M + 1) of vigabatrin and GABA at m/z 130 and 104, respectively, which could be readily monitored. Acetonitrile–water (50:50) was used as the solvent delivery system at a flow rate of 200 μL min^{-1}. Perchloric acid extracts of the brain tissue (10 μL) were injected directly into the mass spectrometer. The stability of vigabatrin and GABA in perchloric acid was established by monitoring their pseudo molecular ions in standard solutions at timed intervals over 24 h. A calibration plot was then established for vigabatrin and GABA over the concentration range 5–1000 ng μL^{-1} using standard solutions in 0.4 M perchloric acid. A series of sample extracts in perchloric acid were then examined consisting of saline blank, vigabatrin, vigabatrin + polysorbate 80, vigabatrin + sodium caprate, and polysorbate 80 + sodium caprate.

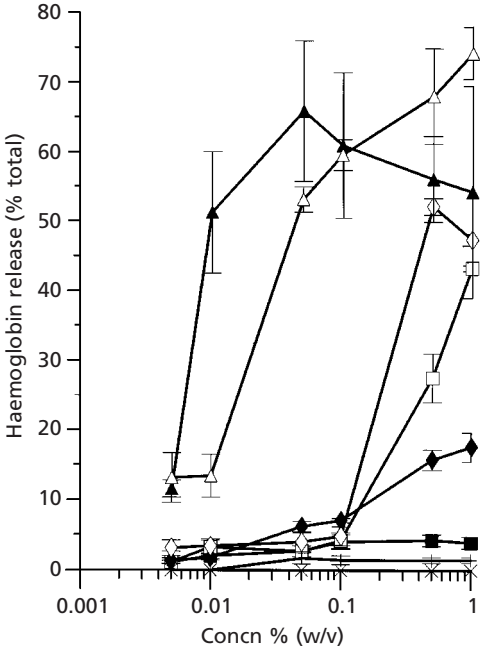

Figure 1 Release of haemoglobin from rat erythrocytes. After 5-min incubations with polysorbate 80 (■), sodium caprate (□) and dextran (×); 2-h incubations with polysorbate 80 (◆), sodium caprate (◇) and dextran (×); and 24-h incubations with polysorbate 80 (▲), sodium caprate (△) and dextran (+). Data are mean ± s.d. of three studies.

In-vivo experiments

Vigabatrin was dissolved in 0.1% polysorbate 80 and 0.1% sodium caprate, and administered intravenously in doses of 4 mL kg^{-1} to male Wistar rats (230–250 g; n = 3). Control rats received 0.9% sodium saline. Rats were killed 2 h after drug and surfactant administration and the brains immediately removed and homogenized in 0.4 M perchloric acid. Each experiment was repeated three times.

The rectal temperature of the rats was monitored at 0, 1, and 2 h.

Statistical analysis

Results are expressed as mean ± s.e.m. The effects of surfactants on vigabatrin absorption and rectal temperature were analysed by one-way analysis of variance followed by a Newman-Keuls test of multiple comparison.

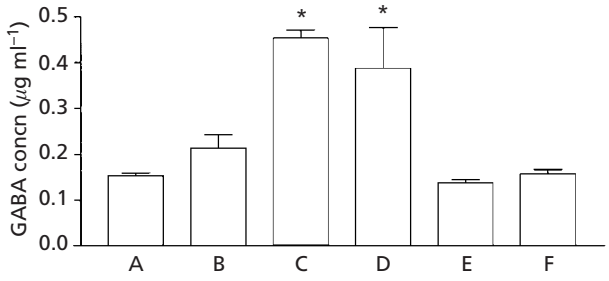

Figure 2 GABA concentrations in rat brains homogenized in 0.4 M perchloric acid. A. 0.9% sodium saline; B. vigabatrin; C. vigabatrin + 0.1% polysorbate 80; D. vigabatrin + 0.1% sodium caprate; E. 0.1% polysorbate 80; F. 0.1% sodium caprate. Data are expressed as $\mu g\ mL^{-1}$ GABA and represent the means ± s.e.m. of values obtained from three rats per group. $*P < 0.01$, significantly different compared with the respective control value, including both 0.9% sodium saline and vigabatrin alone.

Results

Effect of polysorbate 80 and capric acid on red blood cell stability

Before intravenous administration, a range of concentrations of surfactants, polysorbate 80 and sodium caprate, were tested using red blood cell lysis, to establish the non-toxic concentration range (Figure 1). Both polysorbate 80 and sodium caprate displayed some ability to lyse rat erythrocytes in a concentration-dependent manner. After 5 min incubation no lysis was observed at a concentration of 0.1%, but haemolysis had reached 15% after 1 h and 60% after 24 h incubation. These results do not preclude the use of surfactants in-vivo since the dilution factor in the rat blood volume has to be taken into account. This would be greater than one in ten since the blood volume of a rat is on average 65 mL kg^{-1} (Waynforth & Flecknell 1992).

Mass spectrometry analysis of vigabatrin and GABA

Vigabatrin and GABA were shown to be stable in 0.4 M perchloric acid over a period of 24 h. The detection level for vigabatrin and GABA was at least 50 pg and was determined through the series of dilutions. The rat brain extracts, with or without an enhancer, did not show any evidence of the presence of vigabatrin, but GABA was detected and quantified by injection of appropriate GABA standards to provide a calibration. Vigabatrin caused a small, but significant, increase ($P < 0.05$) in whole brain GABA (Figure 2). However, GABA levels

Table 1 Rectal temperature after vigabatrin vehicle administration.

	Time (h)		
	0	1	2
Control (0.9% sodium saline)	38.0±0.10	38.0±0.35	37.9±0.35
Vigabatrin	38.0±0.16	36.8±0.22	35.8±0.42*
Vigabatrin+polysorbate 80	38.2±0.26	34.4±1.24	31.8±0.68†
Vigabatrin+sodium caprate	37.7±0.38	36.6±0.27	31.4±0.67†
Polysorbate 80	38.0±0.11	37.4±0.25	37.4±0.16
Sodium caprate	37.6±0.25	38.1±0.21	37.6±0.31

Data were analysed by one-way analysis of variance followed by Newman-Keuls post-hoc test ($F(5,17) = 28.85$, $P < 0.01$). After 2 h, the rectal temperature was significantly different (†$P < 0.001$) after treatment with vigabatrin + polysorbate 80, and vigabatrin + sodium caprate compared with treatment with sodium saline and vigabatrin alone. There was no difference after 2 h between polysorbate 80 + vigabatrin, and sodium caprate + vigabatrin. *$P < 0.05$ vs sodium saline only.

were higher in the vigabatrin + enhancer samples than in the samples where vigabatrin alone was administered (Figure 2). One-way analysis of variance indicated a significant effect ($F(5,17) = 11.86$, $P < 0.01$) of the surfactants on GABA levels and we must presume vigabatrin absorption. No significant difference was found after treatment with vigabatrin and polysorbate 80 or sodium caprate. Newman-Keuls multiple comparison test showed no difference after the treatment with polysorbate 80 and sodium caprate.

Measurement of rectal temperature

The rectal temperature of the rats is presented in Table 1. Vigabatrin alone decreased rectal temperature by 6%, but this was significantly greater when administered with either polysorbate 80 or sodium caprate (after 2 h). The data were analysed by one-way analysis of variance followed by Newman-Keuls post-hoc test ($F(5,17) = 48.85$, $P < 0.01$). After 2 h the rectal temperature was significantly different after the treatment with polysorbate 80 + vigabatrin, and sodium caprate + vigabatrin compared with treatment with 0.9% sodium saline ($P < 0.001$) and vigabatrin alone ($P < 0.001$). There was no significant difference after 2 h of treatment between polysorbate 80 + vigabatrin, and sodium caprate + vigabatrin. After 1 h there was a significant effect only with polysorbate 80 + vigabatrin compared with 0.9% saline solution ($P < 0.05$).

Discussion

It is generally accepted that many agents designed for brain drug delivery are usually ineffective because of their lack of penetration across the blood–brain and blood–cerebrospinal fluid barriers. The blood–brain barrier is a vital element in the regulation of the constancy of the internal environment of the brain. Electron micrograph studies of the capillaries of the brain reveal that they differ structurally from other capillaries. Brain capillaries are constructed of more densely packed cells and are surrounded by terminations of processes of large numbers of astrocytes and a continuous basement membrane. There are indications that astrocytes produce a substance that influences the capillaries and confers on them the ability to selectively pass various substances, but inhibit others. Substances that cross the blood–brain barrier must fulfill the dual criteria of lipid solubility and a molecular mass less than a threshold of 500 Da (Pardridge 1996). The molecular mass threshold of hydrophilic compounds has been estimated at less than 160 Da (Levin et al 1980). In this study, we examined the effects of the absorption enhancers polysorbate 80 and sodium caprate on the enhancement of vigabatrin absorption into the brain. Although the molecular mass of vigabatrin is 129.16 Da, it seems that high hydrophilicity of vigabatrin is responsible for very low transport across the blood–brain barrier. Vigabatrin acts to increase GABA levels in the presynaptic nerve terminal by inhibiting the activity of GABA transaminase. There is no direct correlation between the blood or brain concentration of vigabatrin and its clinical effect, so monitoring plasma vigabatrin levels is not predictive of a therapeutic response. However, it is possible to relate the activity of vigabatrin to levels of GABA in the brain (Mattson et al 1995). Although we tried to determine the concentration of vigabatrin in the brain homogenate, only GABA was detected. Previous studies on vigabatrin and GABA have used both GC and HPLC. In this study we used a novel and rapid method, selected ion monitoring electrospray mass spectrometry, to estimate vigabatrin and GABA levels directly from perchloric acid extract of the rat brain. The detection level for vigabatrin and GABA was at least 50 pg.

There are a number of techniques which allow the permeability of the blood–brain barrier to be modified. Most of these are non-selective and open the barrier to a range of solutes of varying molecular weight. The most widely used technique is that of osmotic opening, where a short infusion of a hyperosmotic solution,

usually mannitol, is introduced into a carotid artery. Large molecules can be given access to the CNS using this technique and it is therefore thought that mannitol affects the integrity of the tight junctions between the cerebral endothelial cells. One explanation that has been suggested is that the osmotic agent shrinks the endothelial cells and physically opens paracellular pathways. Permeability studies in the rat small intestine, rat colon and Caco-2 cell monolayers indicated that sodium caprate enhances drug absorption by the paracellular route (Sawada et al 1991; Anderberg et al 1993; Lindmark et al 1995; Yamamoto et al 1996). Absorption enhancement mediated by sodium caprate was related to a redistribution of the cytoskeleton, the increase in the intracellular calcium level which might induce contraction of calmodulin-dependent actin filaments followed by structural dilatations in the tight junctions (Hayashi et al 1999). It is probable that the increase of vigabatrin absorption into the brain by sodium caprate is a result of a direct effect altering the paracellular pathways of the blood-brain barrier.

It has been reported that polysorbate 80 may increase the brain level of methotrexate (Azmin et al 1985), or D-kyotorphin (Sakane et al 1989), or as polysorbate 80-coated poly(butyl cynaoacrylate) used in the delivery of dalargin and loperamide across the blood–brain barrier (Alyautdin et al 1995, 1997). The effect of polysorbate 80 on the blood–brain barrier is perhaps based on its direct action on altering the blood–brain barrier. GABA plays an important role in mammalian central thermoregulation, and associated with the biochemical changes after vigabatrin administration (such as sustained elevation in brain GABA concentrations) is a decrease in rectal temperature (Abel & McCandless 1991). In this study we used these two principal parameters to determine if polysorbate 80 or sodium caprate increased CNS penetration of vigabatrin. As previously observed we found that vigabatrin increased brain GABA levels (Qume et al 1995), and also decreased rectal temperature to an extent similar to that seen previously in mice (Schechter & Tranier 1977). The increase in the presence of GABA determined by mass spectrometry corresponded with a decrease in rectal temperature after intravenous administration of vigabatrin alone and together with polysorbate 80 or sodium caprate. Furthermore, the changes in both these parameters were greater when vigabatrin was administered with the absorption enhancers than when given alone. This suggests that polysorbate 80 and sodium caprate increased the penetration of vigabatrin across the blood–brain barrier thereby increasing the effective dose received by the rats. Although not tested in this study, it seems highly probable that such an effect would also raise the convulsant threshold and thereby the anti-epileptic efficacy of vigabatrin. Vigabatrin has been reported to cause concentric visual field loss (Harding et al 2000), as a result of a massive accumulation of retinal GABA. By improving the uptake of vigabatrin into the CNS, it may be possible to reduce the therapeutic dose and thereby lessen this serious side-effect. In the case of other hydrophilic drugs with peripheral side-effects, co-administration of such compounds with absorption enhancers, as a means of decreasing the dose required for a given effect in the CNS, could have considerable therapeutic benefits. Moreover, it is of interest that in spite of clear pharmacological effects of vigabatrin, which could only be mediated at the level of the CNS, we were unable to determine vigabatrin in whole brain. The reason for this is unclear but may reflect the fact that on interaction with GABA-transaminase, vigabatrin can no longer be determined as a separate moiety thereby leaving diminishingly low concentrations of the free drug in the brain, despite the highly sensitive assay used.

In conclusion, both absorption enhancers, polysorbate 80 and sodium caprate, significantly increased the effect of vigabatrin on brain GABA concentrations and produced a decrease in rectal temperature. This strongly suggests that a greater concentration of vigabatrin entered the brain, however we were unable to detect vigabatrin ex-vivo.

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