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Relative Blood–brain Barrier Permeabilities of the Cholecystokinin Receptor Antagonists Devazepide and A-65186 in Rats

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Abstract

The blood–brain barrier permeabilities of the type-A cholecystokinin receptor antagonists devazepide and A-65186 (N_α-3-quinolinoyl-D-Glu-*N*,*N*-dipentylamide) have been compared with those of the reference compounds iodoantipyrine, which readily penetrates the blood–brain barrier, and mannitol, which does not.

Anaesthetized rats received a bolus injection into the left carotid artery of [^{14}C]iodoantipyrine (0·25 μCi) combined with [^{3}H]mannitol, [^{3}H]devazepide or [^{3}H]A-65186 (1 μCi each). Rats were decapitated 12 s after injection and the brains were removed. Four samples of left cerebrum (ca 100 mg each) were solubilized overnight and ^{14}C and ^{3}H activity were measured. The brain-uptake index for each test compound was determined as [(^{3}H/^{14}C for sample)]/[(^{3}H/^{14}C for injectate)] × 100, with a value of 100 representing blood–brain barrier permeability equal to that for iodoantipyrine. The brain-uptake index (mean ± s.e.m.) was 1·6 ± 0·3 for [^{3}H]mannitol (n = 5), 90·6 ± 4·1 for [^{3}H]devazepide (n = 7, $P < 0·001$ compared with mannitol) and 3·5 ± 0·7 for [^{3}H]A-65186 (n = 4, $P > 0·05$ compared with mannitol, $P < 0·001$ compared with devazepide). Thus, devazepide readily penetrated the blood–brain barrier whereas A-65186 did not.

It is concluded that devazepide and A-65186 are likely to be useful pharmacological tools for determining whether cholecystokinin is acting peripherally or at brain sites beyond the blood–brain barrier to produce satiety or any other function mediated by the type A cholecystokinin receptor.

Systemic injection of the type-A cholecystokinin (CCK) receptor antagonist devazepide (also called L-364,718 or MK-329) stimulates food intake in a variety of species (Reidelberger 1994), providing compelling evidence that CCK plays an essential role in mediating the satiety response to ingestion of food. It is not clear from such studies where or how endogenous CCK might act to produce satiety, because CCK is found throughout the brain and in neurocrine and endocrine cells in the gut, because central and peripheral administration of CCK-8 inhibits food intake, and because the cholecystokinin receptor antagonist devazepide readily penetrates the blood–brain barrier (Reidelberger 1994). To investigate further the relative importance of central and peripheral CCK mechanisms in control of feeding, it would be useful to compare the feeding effects of systemic administration of cholecystokinin receptor antagonists that differ in their capacity to cross the blood–brain barrier.

Although numerous cholecystokinin receptor antagonists have been developed, only devazepide has been directly tested for its capacity to penetrate the blood–brain barrier (Pullen & Hodgson 1987). However, because of the hydrophilicity of their chemical structures, several cholecystokinin receptor antagonists have been considered unlikely to cross the blood–brain barrier. These include A-

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65186 (N_α-3-quinolinoyl-D-Glu-*N*,*N*-dipentylamide, also called A-70104; Kerwin 1989), 2-NAP (2-naphthalenesulphonyl-L-aspartyl-2-(phenethyl)-amide; Hull et al 1993), and the CCK analog JMV-179 [*t*-BOC-tyr(SO_3^-)-nle-gly-D-trp-nle-asp-α-2-phenyl-ethyl ester; Lignon et al 1987). A-70104 is the dicyclohexylammonium salt of A-65186; in aqueous solution it is chemically identical with A-65186. In studies examining the effects of these compounds on food intake, neither A-70104 (Ebenezer & Parrott 1993; Castellanos et al 1995) nor 2-NAP (Baldwin et al 1994; Ebenezer & Baldwin 1995) stimulated food intake in pigs or rats when administered under the same conditions under which devazepide stimulated feeding, and at doses that blocked anorexic responses to exogenous CCK. These results suggest that the essential mechanism of CCK action producing satiety requires binding of endogenous CCK to cholecystokinin receptors in brain sites beyond the blood–brain barrier. In contrast, Brenner & Ritter (1995) demonstrated that JMV-179 stimulates food intake in rats, suggesting that CCK acts by a peripheral mechanism to produce satiety. It remains to be determined whether these apparent contradictory results are because the permeability of the brain to the compounds is different.

The aim of this study was to compare blood–brain barrier permeabilities for the cholecystokinin receptor antagonists devazepide and A-65186 with those for iodoantipyrine, which readily penetrates the blood–brain barrier (Sakurada et al 1978), and mannitol, which does not (Oldendorf 1971).

Materials and Methods

Animals

Male Sprague–Dawley rats (Sasco, Kingston, NY; 316–465 g) were provided care according to guidelines established by the Medical Research Service of the Department of Veterans Affairs. The animals had free access to rat chow (Purina #5001) and water and were maintained on a 12-h light–dark cycle with lights off at 1700 h.

Materials

4-Iodo[*N*-methyl-^{14}C]antipyrine ([^{14}C]iodoantipyrine; specific activity (sp. act.) 50·7 mCi $mmol^{-1}$), mannitol-D-[1-[^{3}H](*N*)] ([^{3}H]mannitol; sp. act. 19·7 Ci $mmol^{-1}$) and devazepide-[*N*-methyl-^{3}H] ([^{3}H] devazepide; sp. act. 73·9 Ci $mmol^{-1}$) were purchased from New England Nuclear Life Sciences Products, Boston, MA. The synthesis of *R*-*N*-pentyl-*N*-(4,5-di[^{3}H]pentyl)-N_α-(3-quinolinoyl) glutamic acid amide ([^{3}H]A-65186; sp. act. 92 Ci $mmol^{-1}$) was performed as described by Hulce et al (1998). Briefly, *R*- *N*-(4-pentenyl)- *N*-pentyl-γ-benzyl- N_α-(3-quinolinoyl)glutamic acid amide was prepared by condensation of *N*-(4-pentenyl)-*N*-pentylamine with *R*-γ-benzyl-*N*-BOC-glutamic acid to provide *R*- *N*-(4-pentenyl)-*N*-pentyl-γ-benzyl- N_α-BOC-glutamic acid amide. Subsequent N_α deprotection and condensation with 3-quinoline carboxylic acid provided the desired precursor for tritium incorporation by New England Nuclear Life Sciences Products, Boston MA. [^{3}H]A-65186 was prepared by hydrogenation of the precursor with concomitant hydrogenolysis of the benzyl ester. Chemical (98·9%) and radiochemical (99·6%) purity of [^{3}H]A-65186 were determined chromatographically by the New England Nuclear Life Sciences Products group using authentic, non-radiolabelled A-65186 as standard (provided by Dr James F. Kerwin, Jr, Abbott Laboratories).

Methods

Experiments were performed as described by Oldendorf (1970) and modified by Pullen & Hodgson (1987). Rats were anaesthetized with ketamine hydrochloride (50 mg kg^{-1}). The left common carotid artery was surgically exposed and cannulated with a short length of polyethylene tubing (Intramedic PE-50, 0·58 mm i.d. × 0·965 mm o.d.). Buffer (mM: Na^+ 148, K^+ 4, Ca^{2+} 2, Cl^- 155, buffered to pH 7·4 with 4 mM Tris; 0·25 mL) containing the test compounds was injected rapidly ($< 0·5$ s) into the cannula. The injectate contained 0·25 μCi [^{14}C]iodoantipyrine and 1 μCi [^{3}H]mannitol, 1 μCi [^{3}H]devazepide or 1 μCi [^{3}H]A-65186. Five rats received a co-injection of [^{14}C]iodoantipyrine and [^{3}H]mannitol, seven received a co-injection of [^{14}C]iodoantipyrine and [^{3}H]devazepide, and four received a co-injection of [^{14}C]iodoantipyrine and [^{3}H]A-65186. Rats were decapitated 12 s after injection and the brains were removed. Each of four samples of left cerebrum (approx. 100 mg) was added to a scintillation vial containing Amersham NCS-II tissue solubilizer (1 mL) and incubated overnight at 45°C. The injectate (5 μL) was added to each of four samples of left cerebrum (approx. 100 mg) from a non-injected rat, and treated identically. Scintillation fluid (Ultima Gold, Packard Instrument Co., Meriden CT) (10 mL) and glacial acetic acid (30 μL) were added to each vial. ^{14}C and ^{3}H activity were determined by means of a Packard 1600 TR scintillation counter. Appropriate corrections were made for quench and ^{14}C and ^{3}H crossover.

The brain-uptake index of each tritiated test compound was determined according to equation 1:

$$\text{Brain-uptake index} = [(^3\text{H}/^{14}\text{C for sample})/(^3\text{H}/^{14}\text{C for injectate})] \times 100 \quad (1)$$

Thus the index for a tritiated compound signifies its effectiveness at penetrating the blood–brain barrier compared with that of the reference standard [^{14}C]iodoantipyrine, the movement of which into brain tissue is not impeded by the blood–brain barrier (Sakurada et al 1978).

Data analysis

Brain-uptake index values for each test compound are presented as means ± s.e.m. Data were analyzed by one-factor analysis of variance. Planned comparisons of group means were evaluated by direct contrasts of means by use of the statistical program Systat. Differences were considered significant for $P < 0.05$.

Results and Discussion

Analysis of variance revealed significant differences among the brain-uptake indices of the compounds tested ($F_{2,14} = 238$, $P < 0.001$). The index was 1.6 ± 0.3 for [^{3}H]mannitol (n = 5), 90.6 ± 4.1 for [^{3}H]devazepide (n = 7, $P < 0.001$ compared with mannitol) and 3.5 ± 0.7 for [^{3}H]A-65186 (n = 4, $P > 0.05$ compared with mannitol and $P < 0.0001$ compared with devazepide). Thus, the cholecystokinin receptor antagonist [^{3}H]devazepide readily penetrated the blood–brain barrier whereas [^{3}H]mannitol did not. In contrast, the cholecystokinin receptor antagonist [^{3}H]A-65186 was like mannitol and did not readily penetrate the barrier during single capillary passage.

The brain-uptake index of 1.6 ± 0.3 for mannitol is similar to the value of 1.9 ± 0.2 reported by Oldendorf (1971). These findings are consistent with the assumption that the penetration of this non-metabolizable, polar substance through the blood–brain barrier is negligible during a single passage through brain capillaries in anaesthetized rats. Thus, our results with this compound served to establish the presence of a blood–brain barrier in our experimental model.

The brain-uptake index of 90.6 ± 4.1 for devazepide is significantly higher than the value of 68 ± 4 reported by Pullen & Hodgson (1987), who used methods essentially identical with those employed in the current study. We believe that Pullen & Hodgson might have underestimated the brain-uptake index for devazepide because they might not have corrected appropriately for quenching of the reference injectate by brain tissue. Injectate does not seem to have been combined with brain tissue from non-injected rats before addition of tissue solubilizer and counting of samples. Nevertheless, both studies indicate substantial penetration of the blood–brain barrier by the cholecystokinin receptor antagonist devazepide compared with iodoantipyrine, a compound for which brain penetration seems not to be limited by the blood–brain barrier (Sakurada et al 1978).

The brain-uptake index of 3.5 ± 0.7 for A-65186 indicates that unlike devazepide, the penetration of the blood–brain barrier by this cholecystokinin receptor antagonist is negligible. It is therefore concluded that devazepide and A-65186 are likely to be useful pharmacological tools for determining whether CCK is acting peripherally or at brain sites beyond the blood–brain barrier to produce satiety or any other function mediated by the type A cholecystokinin receptor.

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