that the orientation of the dipole in the cyanoguanidine or replacement group in relation to the side chain, and not the dipole moment or its 30° vector, explains most of the variance in the in-vitro H_2 antagonist activity in this series of analogues.

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Penetration of diazepam and the non-peptide CCK antagonist, L-364,718, into rat brain

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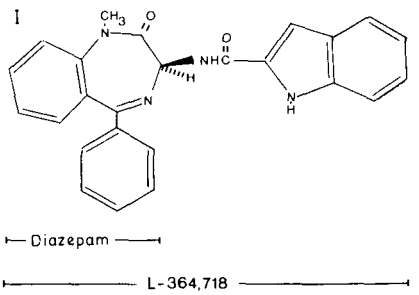
Interest in the actions of the peptide cholecystokinin (CCK) has led to the design and synthesis of several non-peptide CCK antagonists, the most recent being that produced by Merck Sharp & Dohme and designated L-364,718. This can be viewed as modified D-tryptophan linked to diazepam (1-methyl-3-(2-indoloyl)amino-5-phenyl-3*H*-1,4-benzodiazepin-2-one) I.

The compound has an affinity for CCK receptors in the rat pancreas and bovine gallbladder which almost equals CCK itself, and it is an extremely potent CCK antagonist (Chang & Lotti 1986). For example, it will competitively antagonize CCK-induced contractions of the guinea-pig isolated ileum and colon with a pA_2 of 9·9. It will also antagonize the CCK-induced inhibition of gastric emptying in the mouse, with an ED50 of 0·04 mg kg^{-1} (Chang et al 1986; Evans et al 1986).

It has been suggested that CCK may also be a central neurotransmitter (Dockray 1976) and, therefore, the entry of L-364,718 into the brain is of interest since this is a prerequisite for any CNS action. We have investigated the penetration of L-364,718 into brain from blood by measuring its Brain Uptake Index (BUI) as first described by Oldendorf (1970). Since the compound has structural similarities to diazepam (see I) which is known to act on the CNS, we have also measured the BUI for diazepam, for comparative purposes.

Male Wistar rats, 350–500 g were anaesthetized with sodium pentobarbitone (Sagatal, 50 mg kg^{-1} i.p.). The left common carotid artery was surgically exposed and catheterized with a length of Portex pp50 tubing. A 1 mL syringe containing 250 μL of injectate was attached to the tubing. The animal was positioned supine in a guillotine and the injectate administered in a single bolus. The injectate had the composition (mM): Na^+ 148, K^+ 4, Ca^{2+} 2, Cl^- 155, buffered to pH 7·4 with 4 mM Tris. Each 250 μL contained 0·25 μCi of 4-iodo [N-methyl-^{14}C]antipyrine (sp. act. = 55 mCi $mmol^{-1}$, Amersham International), which was the reference solute to which the cerebral capillary was highly permeable, and either 1·0 μCi of [*N*-methyl-^{3}H]diazepam (sp. act. = 80 Ci $mmol^{-1}$, Amersham International) or 1·0 μCi of [*N*-methyl-^{3}H]-L-364,718 (sp. act. = 67 Ci $mmol^{-1}$, Merck Sharp & Dohme) which were test solutes.



Twelve seconds after the administration of the injectate the rat was decapitated and the brain removed. The cerebellum and brain stem were freed from the cerebral hemispheres which were then separated by a single mid-sagittal cut. The left hemisphere was further dissected into cerebral cortex, hippocampus, midbrain and hypothalamus, and striatum according to Glowinski & Iversen (1966). A single tissue sample of about 100 mg was taken from each of these four regions and prepared for scintillation counting by transference to preweighed scintillation vials, which were reweighed, and to each of which was added 1 mL of Amersham NCS tissue solubilizer. The vials were left for 12 h at 45 °C then 10 mL of Amersham OCS scintillant and 30 μL of glacial acetic acid were added to each with shaking. Vials containing 5 μL of injectate were also

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prepared as described above. All samples were counted for radioactivity on a Beckman LS 7500 scintillation counter and appropriate corrections were made for quench and ^{14}C and ^{3}H crossover.

Twenty eight experiments were performed, in 14 [^{3}H]L-364,718 was used as the test solute, in the others [^{14}C]diazepam was used. The BUI for a test solute was determined using the equation:

$$BUI = (\text{counts min}^{-1}_{test}/\text{counts min}^{-1}_{ref})_{brain} \div (\text{counts min}^{-1}_{test}/\text{counts min}^{-1}_{ref})_{injectate}$$

A two-way analysis of variance of the BUI data for [^{3}H]L-364,718 did not show any significant differences either between the 14 experiments or the four brain regions ($P > 0.05$). The same was true for [^{14}C]diazepam data ($P > 0.05$) (Table 1). In view of this the four brain region values were pooled to produce a single mean left-hemisphere value for each experiment. The average of these for [^{3}H]L-364,718 was 0·76 ± 0·03 (mean ± s.e.m.), n = 14. The corresponding average for [^{14}C]diazepam was 1·17 ± 0·05, n = 14. These two values are significantly different ($P < 0.001$, Student's t-test).

Table 1. Brain Uptake Index values for L-364,718 and diazepam.

	L-364,718			Diazepam		
Brain region	$\bar{X}$	s.e.m.	n	$\bar{X}$	s.e.m.	n
Cerebral cortex	0·68	± 0·04	14	1·17	± 0·09	14
Hippocampus	0·81	± 0·07	14	1·36	± 0·08	14
Midbrain and hypothalamus	0·77	± 0·06	14	1·03	± 0·11	14
Striatum	0·78	± 0·07	14	1·12	± 0·08	14
Whole left hemisphere	0·76	± 0·03	14	1·17	± 0·05	14

When this technique is used the injected bolus of fluid should clear the cerebral vessels of contained blood. However, if significant contamination of the bolus by mixing with blood from cerebral vessels occurs, the measured BUI would be altered. In fact, significant mixing does occur in the vessels supplying the cerebellum, brain stem and the cerebral hemisphere contralateral to the cannulated carotid artery (Oldendorf 1970) and hence, those brain regions were not used to determine BUI data. We used only samples from the cerebral hemisphere on the same side as the cannulated carotid since blood contamination of the bolus is then <8% (Pardridge & Fierer 1985).

As the bolus flows through the brain, the test and reference solutes leave the injectate and enter the tissue at which time the animal is decapitated. The time of decapitation should be such that the brain has been adequately perfused, without allowing solute backflux into the injectate or bolus recirculation. To achieve this a decapitation time between 5–15 s has been suggested (Oldendorf 1970) and hence, our use of 12 s.

Since BUI values are essentially extraction fractions of the test solute measured relative to that of the reference solute, it is necessary to ensure that the reference solute is totally extracted during the experiment, i.e. is a substance to which the cerebral capillary is highly permeable. Therefore, we used [^{14}C]iodoantipyrine (Gjedde et al 1983).

BUI values usually range from 0 (impermeable) to 1·0 (highly permeable), therefore, a value of 1·17 for diazepam demonstrates that it is a drug to which the cerebral capillary is highly permeable. This value, being greater than 1·0, could suggest that diazepam passes through the capillary more rapidly than the reference solute, iodoantipyrine. However, a more likely explanation of our result is that a small amount of brain-to-blood backflux occurred for iodoantipyrine, which did not occur for diazepam, thereby producing the effect of incomplete extraction of iodoantipyrine. This would artificially raise the BUI for diazepam. There are reasons to suppose this occurred since the possibility of backflux in 12 s is very small for diazepam (Gjedde et al 1983) but high for iodoantipyrine (Fenstermacher et al 1981). Even so, a BUI of 1·17 still demonstrates that the entrance of diazepam into the brain from blood is very fast and is limited only by the rate of cerebral blood flow.

A BUI value between 0·1 and 0·9 suggests that solute movement from blood to brain is not only dependent upon blood flow but is also limited by a permeability barrier at the cerebral capillary. Since L-364,718 had a BUI of 0·76 it falls into this category. Although this value is significantly less than that obtained for diazepam, it still indicates a high permeability of the cerebral capillary to this molecule.

These data, therefore, show that it is possible to produce non-peptide ligands for peptide receptors that can easily enter the brain from the blood.

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