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Preferential blockade of cholecystokinin-8S-induced increases in aspartate and glutamate levels by the CCK_B receptor antagonist, L-365,260, in rat brain

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Abstract

In the present studies, the ability of a locally delivered cholecystokinin (CCK) receptor agonist and systemically delivered antagonists to modulate extracellular levels of aspartate and glutamate in the frontal cortex of anaesthetised rats and frontal cortex and caudate-putamen of freely moving rats was investigated using an in vivo microdialysis technique. In the anaesthetised rats, local application of sulphated CCK octapeptide (CCK-8S, 10 μM) into the frontal cortex enhanced extracellular aspartate levels to a maximum of $265 \pm 16\%$ of the basal levels, whereas glutamate levels were increased to a maximum of $168 \pm 7\%$ of the basal levels. Given 40 min prior to the cortical perfusion of 10 μM of CCK-8S, the CCK_B receptor antagonist, L-365,260 (20 mg/kg, s.c.), limited the rise in cortical aspartate by over half to $170 \pm 10\%$ of the basal levels. However, this same dose of L-365,260 still allowed CCK-8S to increase glutamate by $44 \pm 15\%$ above the basal levels. Whereas the enhanced glutamate levels were totally unaffected by systemic administration of the CCK_A receptor antagonist, L-364,718 (20 mg/kg, -40 min, s.c.), this treatment was able to limit the elevation in aspartate to $220 \pm 4\%$ of the basal levels. In the freely moving rats, local perfusion of CCK-8S (10 μM) increased aspartate and glutamate levels to maxima of $275 \pm 12\%$ and $225 \pm 14\%$ of the basal levels, respectively, in the frontal cortex. In the caudate-putamen, aspartate and glutamate levels were also elevated by CCK-8S (10 μM) to $248 \pm 15\%$ and $185 \pm 12\%$ of the basal levels, respectively. The respective increase in aspartate and glutamate induced by CCK-8S (10 μM) were limited to $140 \pm 10\%$ and $124 \pm 6\%$ (frontal cortex), of the basal levels, and $162 \pm 15\%$ and $143 \pm 8\%$ (caudate-putamen), by 40 min pretreatment with L-365,260 (20 mg/kg, s.c.). In conclusion, CCK-8S was able to enhance both aspartate and glutamate overflow in the frontal cortex of anaesthetised rats, and frontal cortex and caudate-putamen of freely moving rats. These increases were preferentially offset by the selective CCK_B receptor antagonist, L-365,260, since no influence could be discerned using the selective CCK_A receptor antagonist, L-364,718. © 1998 Elsevier Science B.V.

Keywords: Aspartate; Glutamate; CCK-8S; L-365,260; L-364,718; Frontal cortex, rat; Caudate-putamen, rat; Microdialysis, in vivo

1. Introduction

There is ample evidence to indicate that cholecystokinin (CCK) is present throughout the brain, released in a K^+ and Ca^{2+}-dependent manner, and may thus act as a neurotransmitter in the central nervous system (CNS, Herrera-Marschitz et al., 1992; Meana et al., 1991; You et al., 1994b). According to the affinity for CCK fragments and antagonist ligands and molecular structure, the CCK receptors can be further divided into two subtypes, designated as CCK_A and CCK_B receptors. The CCK_A receptors exhibit a high affinity for the sulphated CCK octapeptide (CCK-8S), the antagonist, L-364,718 and the C-terminal tetrapeptide (CCK-4), whereas CCK_B receptors are characterised by a relatively high affinity for the agonist, CCK-4 and the antagonist, L-365,260 and a low affinity for L-364,718 (Hill et al., 1987; Boden and Hill, 1988). Using autoradiographic techniques and in situ hybridization, the CCK_B receptors and their mRNA have been found distributed in many brain areas including cerebral cortex, hippocampus, nucleus accumbens, caudate-putamen and thalamus (Hill et al., 1987; Saito et al., 1980; Wank et al., 1992; Vanderhaeghen and Schiffmann, 1992). On the other

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hand, CCK_A receptors have been detected in the interpeduncular nucleus, ventral tegmental area, substantia nigra, area postrema and nucleus tractus solitarius (Hill et al., 1987; Wank et al., 1992; Vanderhaeghen and Schiffmann, 1992), although this subtype of receptor may be more widely distributed than is generally believed (Mercer and Beart, 1997).

The relatively high concentration of CCK receptors in areas of the limbic system including the hippocampus, amygdala, temporal cortex, ventral tegmental area, nucleus accumbens and frontal cortex (Dockray, 1980; Goltermann et al., 1980), imply that CCK receptors may be involved in psychiatric disorders such as schizophrenia (Rupniak, 1992) and anxiety (Costall et al., 1991; Vasar et al., 1992). How they interact with neurotransmitters is not clear, although evidence has already accumulated to show that CCK receptor ligands can modulate dopamine release (Rupniak, 1992; Corwin et al., 1995; You et al., 1996), γ-aminobutyric acid (GABA) release (De Belleroche and Bandopadhyay, 1992) and levels of excitatory amino acids within the rat brain (You et al., 1994a,b, 1996; Freedman et al., 1994; Godukhin et al., 1995).

In this latter regard, CCK has been shown to co-exist with glutamate in neurones of several brain regions and to facilitate neuronal excitation (Boden and Hill, 1988; Kelly and Larkman, 1989), which may be associated with the activation of protein kinase C (Kelly and Larkman, 1989), opening K^+ conductance pathways, hyperpolarising the plasma membrane and evoking intracellular Ca^{2+} release. Indeed, neuroanatomical studies have revealed that some populations of cortico-striatal glutamatergic neurones contain CCK-8 (Burgunder and Young, 1990; Morino et al., 1992), and their terminals may thus provide the source of endogenous CCK that can influence neurotransmitter release within the caudate-putamen. In previous reports, local application of CCK-8S was observed to enhance aspartate and glutamate release in the brain of anaesthetised (e.g., You et al., 1994b, 1996; Godukhin et al., 1995) and freely-moving rats (Freedman et al., 1994). In the present studies, we further investigated the effects of central perfusion of CCK-8S on aspartate and glutamate release in the frontal cortex of anaesthetised rats and the frontal cortex and caudate-putamen of freely moving rats using an intracerebral microdialysis technique. Preliminary findings have been presented to the British Pharmacological Society (Ge et al., 1997a,b).

2. Materials and methods

2.1. Animals

Male Wistar rats (200–250 g; Bristol-bred) were housed in groups of 6 in a temperature (22 ± 1°C)–humidity-controlled environment with a 12 h light–dark cycle (light on 8:00) and free access to food and water. All procedures were performed in strict accordance with the guidelines, and approval by the Animals (Scientific Procedures) Act of 1986.

2.2. Assessment of extracellular amino acid levels in the frontal cortex of anaesthetised rats

Rats were anaesthetised with chloral hydrate (400 mg/kg, i.p.) and fixed onto a stereotaxic frame. A custombuilt microdialysis probe (4 mm AN69 dialysis membrane, external/internal diameter 310/220 μm, molecular weight cutoff 40 000; Hospal Medical) was gently implanted into the frontal cortex of the right hemisphere (final microdialysis probe location, mm, A +3.5, −L 1.5, V −5.5, relative to bregma, Paxinos and Watson, 1986) and perfused with an artificial cerebrospinal fluid (aCSF; mM: NaCl 126.6, KCl 2.4, KH_2PO_4 0.49, $MgCl_2$ 1.28, $CaCl_2$ 1.1, $NaHCO_3$ 27.4, Na_2PO_4 0.48, glucose 7.1, initial pH 7.4) at 2 μl/min. Dialysate samples collected for at least the first 100 min were discarded, and subsequent samples were collected every 20 min. After the establishment of a reproducible baseline of dialysate aspartate and glutamate levels, drugs (or vehicle) were administered either via the perfusing aCSF or systemically. Dialysate aspartate and glutamate levels (as well as those of serine, glycine, arginine, taurine and tyrosine) were quantified immediately by high-performance liquid chromatography coupled to a fluorescence detector as detailed below. The body temperature of the rats was maintained at 37°C using Homeothermic Blanket control Unit and 100 mg kg^{-1} h^{-1} of chloral hydrate were injected to maintain the rats in surgically anaesthetised status.

At the end of each experiment, microdialysis probe placement was verified visually by coronal slicing of the brain with a freezing microtome. Data from animals, where the microdialysis probes were not correctly located within the frontal cortex, were not included in the present report.

2.3. Assessment of extracellular amino acid levels in the frontal cortex and caudate-putamen of freely moving rats

Rats were anaesthetised with chloral hydrate (400 mg/kg, i.p.) before 10-mm long guide cannulae (19-gauge stainless steel tubing; Coopers Needle Works, Birmingham) were stereotaxically inserted. The tips of the indwelling guide cannulae were overlying the frontal cortex by 1–2 mm (for frontal cortex) or rested in the cortex overlying the caudate-putamen (for caudate-putamen) and the cannulae were secured to the skull with screws and dental cement.

At least 24 h after stereotaxic location of the guide cannulae, rats were immobilised using a soft-cloth wrapping technique. Microdialysis probes (see Section 2.2 for details) were gently inserted into the frontal cortex of the right hemisphere (final microdialysis probe tip location, mm, A +3.5, −L 1.5, V −5.5, relative to bregma,

Paxinos and Watson, 1986) and the caudate-putamen of the left hemisphere (final microdialysis probe tip location, mm, A +0.5, L −2.5, V 6.8, relative to bregma, Paxinos and Watson, 1986) and fixed using cyanoacrylate glue, and individually placed in a single Perspex experimental animal cage (with free access to food and water). Both probes were perfused with aCSF at 2 μl/min. Dialysate samples collected for at least the first 100 min were discarded and subsequent samples were collected every 20 min. After the establishment of a reproducible baseline of dialysate aspartate and glutamate levels, drugs (or vehicle) were administered either via the perfusing aCSF or systemically. Dialysate aspartate and glutamate levels (as well as those of serine, glycine, arginine, taurine and tyrosine) were quantified immediately by high-performance liquid chromatography coupled to a fluorescence detector.

At the end of each experiment, microdialysis probe placement was verified visually by coronal slicing of the brain with a freezing microtome. Data from animals in which the microdialysis probes were not correctly located within the frontal cortex were not included in the present report.

2.4. High-performance liquid chromatography (HPLC) system for the quantification of amino acids

For determination of amino acid levels in the dialysates, the HPLC system was based on a binary gradient system (Gilson Medical Electronics, Villiers-le-Bel, France) as detailed previously (Kilpatrick and Mozley, 1986; Kilpatrick, 1991). Briefly, two model 303 single-piston pumps were electrically and hydraulically connected to a model 802C manometer/pulse dampener. Solvent mixing was achieved in-line with a model 811 dynamic mixer housing a 1.5-ml mixing chamber and connected to an analytical column (Ultratechsphere, 5 μm, ODS; 250 × 3.2 mm; HPLC Technology, Macclesfield, UK). The eluate from the analytical column was passed into a fluorescence detector (HP 1046A; Hewlett-Packard) with an excitation wavelength at 231 nm and the emission wavelength at 450 nm. The dialysates were derivatised pre-column with *ortho*-phthaldialdehyde and β-mercaptoethanol in a potassium borate buffer at pH 9.85.

The optimised mobile phase consisted of two solvents. Solvent A comprised a 0.075-M KH_2PO_4 solution containing 10% v/v methanol with an apparent pH of 6.2, and solvent B was 100% methanol. The mobile phase was delivered to the analytical column at a rate of 0.5 ml/min (for details see Kilpatrick and Mozley, 1986; Kilpatrick, 1991).

2.5. Data analysis

To analyse the microdialysis data, two-way ANOVA (analysis of variance) (time vs. treatment) followed by Dunnett's *t*-test was used for all experiments. A *p*-value of 0.05 or less was required for significance.

2.6. Drugs

L-365,260 (3*R*(+)-*N*-(2,3-dihydro-L-methyl-2-*oxo*-5-phenyl-1H-1,4-benzodiazepine-3-yl)-*N*′-(3-methylphenyl)-
urea; Merck, Sharp and Dohme, Harlow, UK) and L-364,718 (3*S*(−)-*N*-(2,3-dihydro-L-methyl-2-*oxo*-5-phenyl-1H-1,4-benzodiazepine-3-yl)-1H-indole-2-carboxamide; Merck, Sharp and Dohme, Harlow, UK) were dissolved in Labrafil (M 2125 CS; Gattefosse, France). CCK-8S (Bachem, USA) was dissolved in a minimum quantity of distilled water and diluted to the required concentration in aCSF. Methanol was purchased from BDH Laboratory Supplies (Poole, England). All other reagents were obtained from Sigma. All drugs were used as received and were freshly prepared immediately before use.

3. Results

3.1. Basal extracellular levels of aspartate and glutamate in frontal cortex of anaesthetised rats and frontal cortex and caudate-putamen of freely moving rats

The in vitro recoveries of aspartate and glutamate with the homemade microdialysis probes (4 mm dialysis membrane) were 12 ± 2% and 15 ± 1%, respectively (mean ± S.E.M., 12 probes). In both anaesthetised and freely moving rats, the basal extracellular levels of aspartate and glutamate decreased rapidly from the beginning of the experiments and started to stabilise approximately 100 min after perfusion. Only slow decrements from these levels were recorded throughout the time course of the experiments (at least 6 h). In the anaesthetised rats, the respective basal extracellular levels of aspartate and glutamate were 4.36 ± 0.35 and 23.6 ± 3.8 pmol/40 μl (mean ± S.E.M., $n = 15$) in the frontal cortex. In the freely moving rats, the basal extracellular levels of aspartate and glutamate were 1.05 ± 0.12 and 14.7 ± 1.5 pmol/40 μl, respectively, in frontal cortex and 0.69 ± 0.08 and 18.4 ± 0.21 pmol/40 μl, respectively, in caudate-putamen (mean ± S.E.M., $n = 18$). The detection limits for aspartate and glutamate were 10 and 5 fmol, respectively (the ratio of signal to noise equals 3).

3.2. Effects of a CCK receptor agonist and systemic antagonists on extracellular aspartate and glutamate levels in frontal cortex of anaesthetised rats

In the anaesthetised rats, local application of CCK-8S (10 μM; administered via the microdialysis probe) increased the extracellular aspartate and glutamate levels to a maximum of 265 ± 16% ($P < 0.01$, $n = 5$, Fig. 1A) and

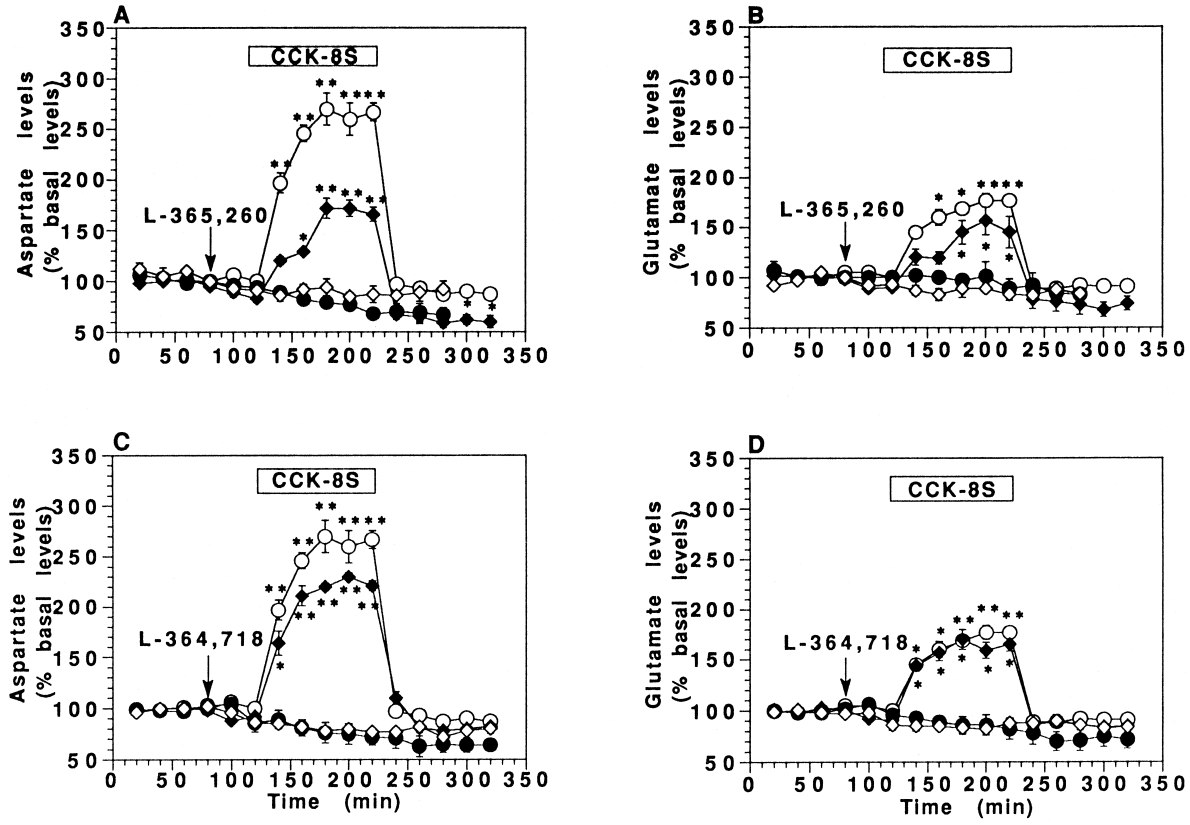

Fig. 1. The influence of CCK-8S, the selective CCK_B receptor antagonist, L-365,260 (A,B) or CCK-8S and the selective CCK_A receptor antagonist, L-364,718 (C,D) on extracellular levels of aspartate (A,C) and glutamate (B,D) in the frontal cortex of anaesthetised rats. In (A) (aspartate levels) and (B) (glutamate levels), the following symbol descriptions apply: ◇ vehicle (Labrafil, 1.0 ml/kg, s.c.), ● L-365,260 (20 mg/kg, s.c., dissolved in Labrafil), ○ vehicle s.c. + CCK-8S (10 μM via probe) and ♦ co-administration of L-365,260 (20 mg/kg, s.c., given 40 min prior to CCK-8S) + CCK-8S (10 μM via probe). In (C) (aspartate levels) and (D) (glutamate levels), the following symbol descriptions apply: ◇ vehicle (Labrafil, 1.0 ml/kg, s.c.), ● L-364,718 (20 mg/kg, s.c., dissolved in Labrafil), ○ vehicle s.c. + CCK-8S (10 μM via probe) and ♦ co-administration of L-364,718 (20 mg/kg, s.c., given 40 min prior to CCK-8S) + CCK-8S (10 μM via probe). Extracellular aspartate and glutamate levels are expressed as a percentage of the mean absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± S.E.M. of 4–7 experiments. Significant changes are indicated as $* P < 0.05$ and $** P < 0.01$ (two-way ANOVA followed by Dunnett's *t*-test).

168 ± 8% ($P < 0.01$, $n = 5$, Fig. 1B), respectively, of the basal levels. The increases in aspartate and glutamate were detectable within 20 min, reached maximal levels within approximately 60 to 80 min and returned to basal levels within one fraction (20 min) when the normal aCSF was restored (Fig. 1A,B). The basal levels of other measured amino acids were (pmol/40 μl): serine, 78 ± 8, glycine, 142 ± 10, arginine, 80 ± 6, taurine, 40 ± 12 and tyrosine, 132 ± 15. Levels of these amino acids were unaltered by perfusion of CCK-8S (10 μM) in this area.

Systemic administration of either of vehicle (Labrafil, 1 ml/kg, s.c.) or the selective CCK_B receptor antagonist, L-365,260 (20 mg/kg, s.c.) alone had no effect on the basal levels of either aspartate or glutamate but L-365,260 (20 mg/kg, s.c.), administered 40 min prior to the cortical perfusion of CCK-8S, limited the increase in aspartate levels induced by CCK-8S by over half to 170% ± 10% of the basal levels (Fig. 1A). Further, this same dose of L-365,260 only allowed CCK-8S (10 μM) to increase glutamate by 44 ± 15% above the basal values in frontal cortex (Fig. 1B).

Systemic administration of either vehicle (Labrafil, 1 ml/kg, s.c.) or the selective CCK_A receptor antagonist, L-364,718 (20 mg/kg, s.c.), had no effect on the basal levels of either aspartate or glutamate in the rat frontal cortex. Given 40 min prior to the cortical perfusion of CCK-8S (10 μM), L-364,718 (20 mg/kg, s.c.) slightly reduced the aspartate levels by approximately 20% from the basal levels (Fig. 1C), but this did not reach statistical significance. Conversely, glutamate levels were totally unaffected by this compound (Fig. 1D).

3.3. Effects of a CCK receptor agonist and antagonists on extracellular aspartate and glutamate levels in the frontal cortex and caudate-putamen of freely moving rats

Intra-cortical application of CCK-8S (10 μM, administered via the microdialysis probe) increased extracellular

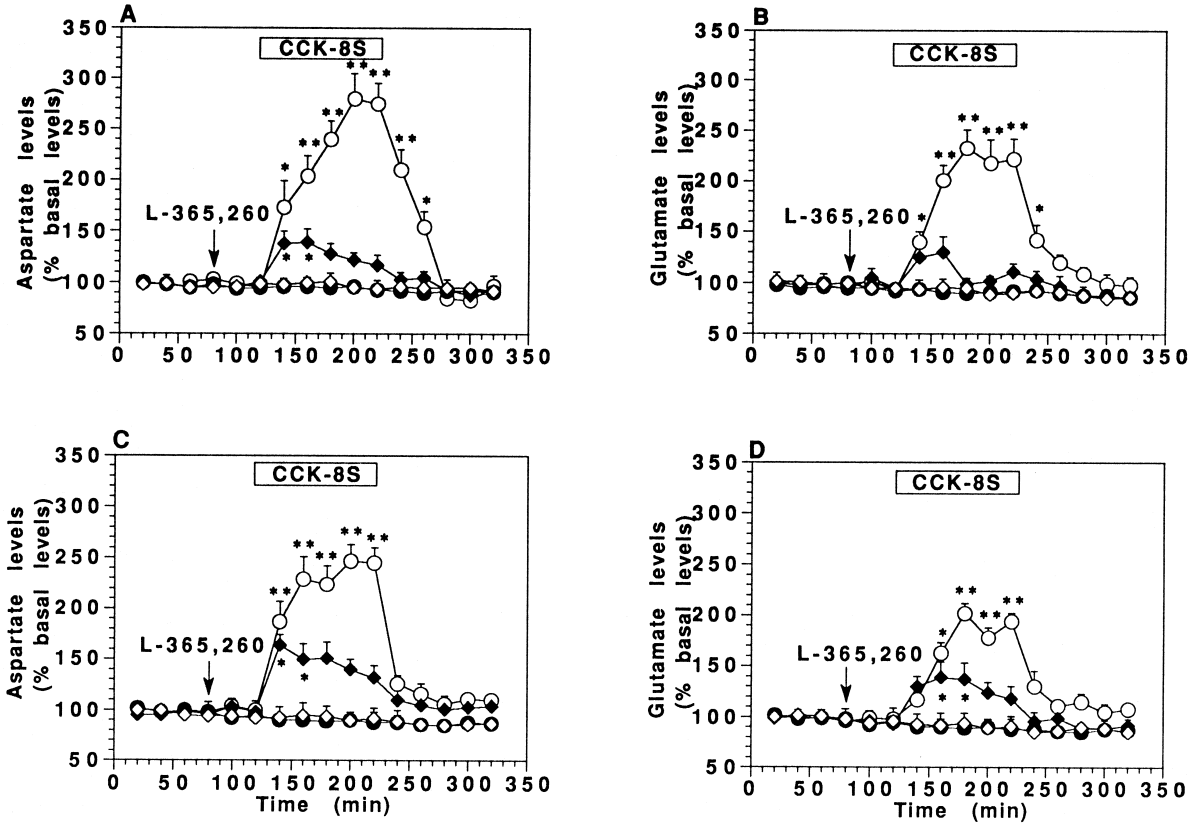

Fig. 2. The influence of CCK-8S and the selective CCK_B receptor antagonist, L-365,260, on extracellular levels of aspartate (A,C) and glutamate (B,D) in the frontal cortex (A,B) and caudate-putamen (C,D) of freely moving rats. In all figures, the following symbol descriptions apply: ◇ vehicle (Labrafil, 1.0 ml/kg, s.c.), ● L-365,260 (20 mg/kg, s.c., dissolved in Labrafil), ○ vehicle s.c. + CCK-8S (10 μM via probe) and ♦ co-administration of L-365,260 (20 mg/kg, s.c., given 40 min prior to CCK-8S) + CCK-8S (10 μM via probe). Extracellular aspartate and glutamate levels are expressed as a percentage of the mean absolute amount in the 4 collections preceding the drug treatment. Data represent the mean ± S.E.M. of 5–7 experiments. Significant changes are indicated as $* P < 0.05$ and $** P < 0.01$ (two-way ANOVA followed by Dunnett's *t*-test).

levels of aspartate and glutamate to a maximum of 275 ± 12% and 225 ± 14% of the basal levels, respectively, in the frontal cortex of freely moving rats (Fig. 2A,B) while in the caudate-putamen, intra-striatal CCK-8S enhanced the aspartate and glutamate levels to a maximum of 248 ± 15% and 185 ± 12% of the basal levels, respectively (Fig. 2C,D). Levels of other amino acids such as serine, glycine, arginine, taurine and tyrosine were unaltered by CCK-8S (10 μM) in either area. The basal levels of other measured amino acids were (pmol/40 μl): in frontal cortex; serine, 58 ± 4, glycine, 89 ± 10, arginine, 53 ± 5, taurine, 15 ± 2 and tyrosine, 38 ± 3; in caudate-putamen; serine, 158 ± 12, glycine, 138 ± 8, arginine, 125 ± 8, taurine, 49 ± 5 and tyrosine, 159 ± 15. Levels of these amino acids were unaltered by perfusion of CCK-8S (10 μM) in both areas.

Systemic administration of either vehicle (Labrafil, 1 ml/kg, s.c.) or the selective CCK_B receptor antagonist, L-365,260 (20 mg/kg, s.c.) alone, had no effect on the basal levels of either aspartate or glutamate in the rat frontal cortex and caudate-putamen. Given 40 min prior to the cortical perfusion of CCK-8S (10 μM), L-365,260 (20 mg/kg, s.c.) limited the increases in aspartate and glutamate levels induced by CCK-8S to 140 ± 10% and 124 ± 6% of the basal levels, respectively, in the rat frontal cortex (Fig. 2A,B), and 162 ± 15% and 143 ± 8%, respectively, in the caudate-putamen (Fig. 2C,D).

4. Discussion

In the present studies, we used intracerebral microdialysis to investigate the effects of central perfusion of CCK-8S on extracellular levels of various amino acids, especially aspartate and glutamate, in the frontal cortex and caudate-putamen of anaesthetised and freely moving rats. The present results have demonstrated that local administration of 10 μM CCK-8S provokes a robust elevation of extracellular levels of both aspartate and glutamate in frontal cortex of anaesthetised rats and in the frontal cortex and caudate-putamen of their freely moving counterparts. These increases in extracellular excitatory amino acids were (i) time-locked to the CCK-8S infusion and (ii) preferentially prevented (though to differing extents) by the selective CCK_B receptor antagonist, L-365,260. Essentially no effects were observed in either area with the use of the selective CCK_A receptor antagonist L-364,718.

The present findings concur with previous reports concerning (i) basal levels of extracellular aspartate and glutamate in the rat frontal cortex under anaesthesia, (ii) CCK-8S-evoked increases in aspartate and glutamate release within this tissue under anaesthetised conditions, and (iii) the sensitivity of this action of CCK-8S to antagonism by systemic pretreatment with the selective CCK_B receptor antagonist, L-365,260 (e.g., You et al., 1994a, 1996; Godukhin et al., 1995). Our data provide the time course of action of CCK-8S and show that the elevations in sampled excitatory amino acids are essentially time-locked to the infusion of CCK-8S, persisting for at least the duration of these experiments (100 min infusion). This implies that if the responses are receptor-mediated, such receptors do not readily desensitise. In view of the ability of L-365,260 to offset the actions of CCK-8S, there is currently no reason to believe that these responses are not response-mediated and the receptors involved are likely to be of the CCK_B subtype. Indeed, it is generally accepted that the predominant CCK receptor in the rodent CNS is of the CCK_B subtypes (Hill et al., 1987, 1990; Saito et al., 1980; Wank et al., 1992; Vanderhaeghen and Schiffmann, 1992) and perhaps not surprisingly, most of the behavioural and functional effects of CCK are likely to be related to its interaction with CCK_B receptors in the CNS (Hill et al., 1987; Wank et al., 1992; Vanderhaeghen and Schiffmann, 1992). A small but non-significant, inhibitory action of the CCK_A antagonist, L-364,718, on CCK-8S-evoked aspartate levels in the frontal cortex is in agreement with data from others (e.g., You et al., 1994a, 1996; Godukhin et al., 1995). This probably reflects the relative paucity of CCK_A receptors within the rodent CNS and especially in the prefrontal cortex (Hill et al., 1987; Wank et al., 1992; Vanderhaeghen and Schiffmann, 1992), although this subtype of receptor may be more widely distributed than is generally believed (Mercer and Beart, 1997). However, the possibility of the different anaesthesia used in the present studies (chloral hydrate) and the previous report (halothane, You et al., 1994a; Godukhin et al., 1995) to cause such discrepancy cannot be ruled out.

All except one (Freedman et al., 1994) reported investigation on the influence of CCK ligands on extracellular levels of amino acids have been performed under anaesthetised conditions (You et al., 1994a,b, 1996; Godukhin et al., 1995). The effects of these ligands in freely moving rats have not yet been fully investigated, and part of the purpose of the present study was to examine whether differences in extracellular amino acids and their modulation existed between the anaesthetised and freely moving rats. In comparison with the anaesthetised rats, the basal levels of amino acids measured in both regions were lower in the freely moving rats. In these animals, central perfusion of identical concentrations of CCK-8S caused somewhat greater increases in frontal cortex glutamate and aspartate levels (approximately 10% higher for aspartate and 60% higher for glutamate) than those obtained from anaesthetised rats. However, in both anaesthetised and freely moving groups, systemic administration of the same dose of L-365,260 caused similar inhibition of the CCK-8S-induced increases in aspartate and glutamate levels. In the caudate-putamen, the CCK-8S-evoked increases in aspartate and glutamate were much lower, and the inhibitory effects of L-365,260 on the CCK-8S-induced response were less potent than those obtained in the frontal cortex. The mechanisms behind such regional variation are not clear, although such results may imply that numbers of the CCK receptor (most likely of the CCK_B subtype) and/or their effector coupling are relatively lower in the caudate-putamen than in the cortex (Burgunder and Young, 1990; Savasta et al., 1988). Nevertheless, an effective blockade of CCK-8S-induced aspartate release in the caudate-putamen of freely moving animals by systemic L-365,260 has previously been reported (Freedman et al., 1994).

We have no evidence to pinpoint the release sites of aspartate and glutamate within the cortex or caudate-putamen. However, glutamate (and possibly aspartate as well), has long been considered to be the principal neurotransmitter within cortico-striatal pathways (Fonnum et al., 1981; Druce et al., 1982; Girault et al., 1986) and CCK may co-exist with an excitatory amino acid in these neurones. For example, using in situ hybridization, a large proportion of cortical neurones have been found to express CCK mRNA (Burgunder and Young, 1990; Savasta et al., 1988) and a number of these CCK mRNA-containing neurones project to the caudate-putamen (Burgunder and Young, 1990). Further, in an attempt to destroy cortico-striatal fibres, a lesion placed in the frontal cortex had no effect on basal extracellular levels of CCK, aspartate and glutamate in the caudate-putamen, but did reduce the K^+-evoked increases in CCK, aspartate and glutamate in this deafferented area (Herrera-Marschitz et al., 1992). It is likely that many CCK_B receptors are localised to interneurones whose activation could eventually lead to a release of aspartate and/or glutamate. Recently, there is a report indicating that decortication and callosotomy can produce a decrease in CCK and glutamate (You et al., 1994a); therefore, further studies are needed to reveal the mechanisms of release of aspartate and glutamate and interaction with CCK receptors in the CNS.

Since most of the CCK mRNA-containing neurones have been identified in dopamine containing areas, such as caudate-putamen, nucleus accumbens and substantia nigra, an interaction of CCK with dopamine neurones should also not be discounted. Indeed, CCK receptor ligands have been shown to modulate dopamine release in rat brain (e.g., Corwin et al., 1995; Marshall et al., 1991), which may thus indirectly modify glutamate/aspartate release.

The physiological significance of the effects of CCK-8S on extracellular aspartate and glutamate levels are not yet understood. There is increasingly evidence to link CCK receptors with psychiatric disease such as anxiety (e.g., Costall et al., 1991; Vasar et al., 1992; Webb et al., 1996)

and schizophrenia (e.g., Rupniak, 1992). Most recently, the excitatory amino acids such as aspartate and glutamate have also been implicated in the control of psychiatric disease (e.g., Olney and Farber, 1995; Monn et al., 1997). Therefore, the increases of aspartate and glutamate release in the rat brain may be a useful index for further investigation in psychiatric disorders, and the ability of CCK-8S to elevate these excitatory amino acids should provide a fairly robust CNS tested for activity at CCK_B receptors.

In summary, local application of CCK-8S increased extracellular levels of aspartate and glutamate in the frontal cortex and caudate-putamen of both anaesthetised and freely-moving rats. Such responses were preferentially prevented by 40 min pretreatment of the selective CCK_B receptor antagonist, L-365,260.

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