J. Pharm. Pharmacol. 1988, 40: 111–115
Received June 10, 1987

Interaction of Memantine with Cholecystokinin Receptors in Mouse Brain

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Abstract—The effect of memantine on CCK receptors in mouse brain has been investigated using particles of dissected cortex and striatum. Total binding of radio-labelled CCK_{33} was one-half maximal within 10 min of incubation and reached a maximum after 30 to 60 min when either cortex or striatum was used. Non-specific binding (presence of 100 μM unlabelled CCK_8) was 50 to 80% of total binding at steady state conditions. CCK_8 inhibited specific binding of radiolabelled CCK_{33} in a dose-dependent manner; the IC50 (half-maximal inhibitory concentration) was in the range 3 to 4 nM. Memantine increased CCK binding in a concentration-dependent manner, though at high concentrations. The EC50 (half-maximal effective concentration) of this effect was <100 μM. The memantine effect is not due to an inhibition of labelled CCK degradation in the medium. The effect of memantine on CCK binding is unique for brain since it was not observed in pancreatic acinar membranes. These data, therefore, suggest a modulatory effect of memantine on CCK receptors in mouse brain (cortex and striatum) particles.

A number of studies have been carried out characterizing high affinity CCK binding sites in brain, both by ligand binding to membrane particles and by tissue section autoradiography (Gaudreau et al 1983; Hays et al 1980; Innis & Snyder 1980; Praissman et al 1983; Saito et al 1980; Van Dijk et al 1984; Zarbin et al 1983). In the rat and mouse brain, the regions yielding the highest specific binding are the cerebral cortex, olfactory bulb, caudate, hypothalamus and hippocampus (Gaudreau et al 1983; Saito et al 1980; Saito et al 1981a; Van Dijk et al 1984). By using antisera specific for the carboxyl terminal of the CCK molecule, immunoreactive material has been found in nerves, fibres and cell bodies of the brain (Larsson & Rehfeld 1979; Straus et al 1977; Innis et al 1979; Loren et al 1979). Radioimmunoassay measurements have also shown that CCK is not equally distributed throughout the rodent brain; the highest concentrations are found in the cerebral cortex, with lower levels in the hippocampus, olfactory lobes, caudate nucleus, and hypothalamus (Rehfeld 1978; Dockray et al 1978; Schneider et al 1979).

CCK is presumed to function as a neurotransmitter or neuromodulator (Williams 1982; Dockray 1982). There appears to be a link between CCK and dopamine; first, CCK_8 antagonizes the dopamine-induced hyperactivity (Itoh & Katsuura 1981; Takeda et al 1986; Van Ree et al 1983), second, CCK appears to stimulate the release of dopamine in neurons in the midbrain (Skirboll et al 1981), and third, a subpopulation of mesencephalic CCK-containing neurons also contains dopamine; these neurons appear to run from the ventral tegmentum to the caudal medial nucleus accumbens (Hökfelt et al 1980a). Lesioning these CCK-dopamine neurons with 6-hydroxydopamine resulted in both an increase in CCK receptors (Chang et al 1983) and a loss of CCK and tyrosine hydroxylase immunoreactivity (Hökfelt et al 1980a, b). Both the co-location of CCK and dopamine in neurons, particularly in the substantia nigra, and the involvement of CCK receptors in Huntington's disease does suggest several roles of CCK, e.g. in extrapyramidal motor function, the aetiology of Parkinson's disease and schizophrenia (Hökfelt et al 1980a).

Memantine has dopaminomimetic properties (Maj 1982; Wesemann et al 1983), enhances the locomotor activity (Youssif & Ammon 1986) and is clinically used in the therapy of neurogenic motor diseases (e.g. spasticity) and Parkinson's disease (Fischer et al 1977). Therefore, the interaction of memantine with CCK binding sites was investigated in mouse brain.

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Materials and Methods

Animals

Adult male Swiss-Webster mice were maintained at 24 °C on alternating 12 h light–dark cycles and provided with free access to appropriate chow and water.

Chemicals

Pure natural porcine CCK_{33} was obtained from the Gastrointestinal Hormone Laboratory, Karolinska Institute, Stockholm, Sweden. Synthetic CCK_8 was from the Squibb Institute for Medical Research, Princeton, NJ. Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) and bacitracin were from Sigma Chemical Co., St Louis, Mo, and bovine serum albumin (BSA) fraction V from Miles Laboratories (Elkhart, IN). *N*-succinimidyl 3-(4-hydroxy-[5-^{125}I]iodophenyl) propionate ([^{125}I]Bolton Hunter reagent) was from New England Nuclear, Boston, MA. CCK_{33} was iodinated to specific activity of 200–300 μCi μg^{-1} by conjugation with [^{125}I]Bolton Hunter reagent, according to the method of Sankaran et al (1979).

Memantine (Akatinol) was from Merz & Co., Frankfurt, West Germany. All other chemicals and reagents of analytical grade were obtained from Sigma Chemical Co., St Louis, Mo.

Methods

Preparation of brain particles was identical to previously described methods with all procedures carried out at 4 °C (Saito et al 1980, 1981a). Briefly, animals were not fasted since fasting has been shown to increase CCK binding in some brain areas (Saito et al 1981b). The animals were decapitated between 0930 and 1000h, their brains removed and placed in ice-cold 0·9% NaCl. The brain was dissected on ice and cerebral cortex and striatum were homogenized in 10 volumes of 50 mM Tris buffer (pH 7·4) at 4 °C by a motor-driven Teflon-glass homogenizer (Saito et al 1980). The homogenate was centrifuged at 40 000*g* for 15 min at 4 °C, the pellet resuspended in the same volume of Tris buffer, similarly recentrifuged and then resuspended in binding buffer. Membrane protein was determined by the BioRad protein assay (BioRad Laboratories, Richmond, CA). Membranes were stored frozen in binding buffer at −70 °C without loss of activity for four weeks.

Plasma membranes of isolated pancreatic acini were prepared using Swiss-Webster male mice. Krebs-Henseleit bicarbonate buffer containing 0·1 mg mL^{-1} of purified collagenase, 0·1 mg mL^{-1} of soybean trypsin inhibitor (SBTI), and 2 mg mL^{-1} of bovine serum albumin (BSA) was injected into pancreatic parenchyma and the tissue was incubated with shaking at 37 °C for 50 min. The acini were then dissociated mechanically and purified by centrifugation through buffer containing 4% BSA. Subsequent operations were carried out at 4 °C. The acini were homogenized by use of a Teflon-glass homogenizer in 0·3 M sucrose buffered with 10 mM Hepes (pH 7·4), containing 1 mM benzamidine. After centrifugation at 1000*g* for 10 min, the pellet was resuspended in the aforementioned Hepes buffer enriched with 1·35 M sucrose. This suspension was overlaid with the 0·3 M sucrose solution and centrifuged at 190 000*g* for 120 min. The plasma membrane-enriched fraction collected from the interface was pelleted and resuspended in a buffer of pH 7·0 containing 10 mM Hepes, 1 mM EGTA, 5 mM $MgCl_2$, 1 mM benzamidine, and 0·2 mM phenylmethylsulphonyl fluoride (PMSF). Protein concentration was measured by the Lowry assay and the membrane preparation was frozen at −70 °C.

Standard binding buffer for brain receptors consisted of 118 mM NaCl, 4·7 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, 10 mM Hepes, 5 mg mL^{-1} bovine serum albumin and 0·5 mg mL^{-1} bacitracin and was adjusted to pH 6·5. The binding buffer for acinar membranes was the same except that 1 mg mL^{-1} bacitracin, 0·2 mg mL^{-1} soybean trypsin inhibitor and pH 7·0 were used.

Binding experiments were performed as previously described (Saito et al 1981a) in a total volume of 150 µL, in polyethylene microcentrifuge tubes along with brain particles or acinar membranes. The final protein concentration was 0·6 and 0·05 mg mL^{-1}, respectively; 50 pM $[^{125}I]CCK_{33}$ was added and non-saturable binding was determined in the presence of 100 µM and 100 nM CCK_8, respectively. Incubations were performed at 24 °C, and bound and free ligand were separated by centrifugation at 10 000*g* in a Beckman 152 microcentrifuge. The pellets were rinsed twice with cold buffer, recentrifuged, the tips of the tubes cut off and radioactivity and protein content determined. Degradation of $[^{125}I]CCK_{33}$ was assessed by precipitation with 10% (w/v) trichloroacetic acid (final concentration).

Comparing rats and mice, preliminary experiments with cortex and striatum particles showed that the major part (74 to 83%) of total binding in rat brain particles resulted from non-specific binding which confirms recent data of others (Williams et al 1986). Therefore, all the experiments shown were performed with mice.

Results

Initial experiments showed that binding of $[^{125}I]CCK_{33}$ to mouse brain particles was maximal at 24 °C; total binding reached a steady state after 30 to 60 min (Figs 1, 2). Experiments at 37 °C showed a rapid binding and a decline thereafter (no steady state; data not shown). All experiments, therefore, were performed at 24 °C.

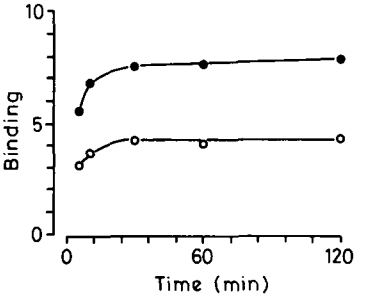

FIG. 1. Time course of [^{125}I]CCK binding to mouse cortex. $[^{125}I]CCK_{33}$ (50 pM) was incubated with mouse cortex particles in the absence (● total binding) and presence of 100 µM unlabelled CCK_8 (○ non-specific binding). Values are given as per cent bound radioactivity per 100 µg protein and are the mean of three individual experiments run as triplicates.

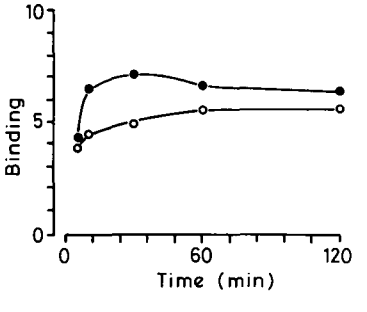

FIG. 2. Time course of [^{125}I]CCK binding to mouse striatum. $[^{125}I]CCK_{33}$ (50 pM) was incubated with mouse striatum particles in the absence (● total binding) and presence of 100 µM unlabelled CCK_8 (○ non-specific binding). Values are given as per cent bound radioactivity per 100 µg protein and are the mean of three individual experiments run as triplicates.

In Figs 1 and 2 total and non-specific binding of $[^{125}I]CCK_{33}$ to mouse brain particles are shown. In either type of experiment non-specific binding was 4–5% of totally added radioactivity per 100 µg protein after at

least 10 min. At steady state (between 30 and 60 min) specific binding (total binding minus non-specific binding) was between 0·6 and 3·6% of totally added radioactivity per 100 μg protein depending on the brain region used (cortex > striatum). All further experiments were performed at 45 min (after reaching a steady state).

As shown in Figs 3 and 4 unlabelled CCK_8 was able to inhibit specific [^{125}I]CCK_{33} binding to mouse cortex and striatum in a dose-related manner. The IC50s of this effect were in the range 3–4 nM. Memantine increased [^{125}I]CCK_{33} binding in a concentration-dependent manner, though at high concentrations (Figs 3, 4). The IC50s of this effect were in the range 100–200 μM.

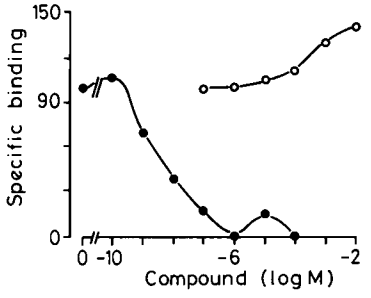

FIG. 3. Effect of (●) unlabelled CCK_8 and (○) memantine on specific [^{125}I]CCK_{33} binding to mouse cortex. [^{125}I]CCK_{33} (50 pM) was incubated for 45 min with mouse cortex particles plus increasing concentrations of unlabelled CCK_8 or memantine. Values are given as per cent maximally bound in the absence of unlabelled CCK_8 and are the mean of three individual experiments run as duplicates.

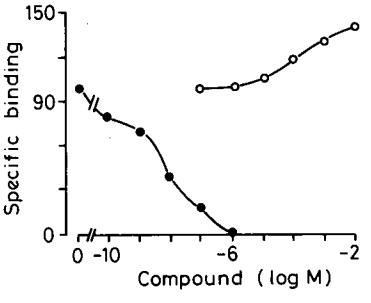

FIG. 4. Effect of (●) unlabelled CCK_8 and (○) memantine on specific [^{125}I]CCK_{33} binding to mouse striatum. [^{125}I]CCK_{33} (50 pM) was incubated for 45 min with mouse striatum particles plus increasing concentrations of unlabelled CCK_8 or memantine. Values are given as per cent maximally bound in the absence of unlabelled CCK_8 and are the mean of three individual experiments run as duplicates.

The incubation medium was checked for degradation of labelled material. The results are shown in Table 1. 2·7 ± 0·3% of [^{125}I]CCK_{33} was already degraded at the beginning of the experiments (time point zero). A small increase in degraded material was observed in the incubation medium at the end of the 45 min incubation period (Table 1; no memantine added). High concentrations of memantine (1 and 10 mM) present for 45 min in the incubation medium did not further change the degradation of [^{125}I]CCK_{33} in the medium when either mouse cortex or striatum was used (Table 1).

Table 1. Degradation of [^{125}I]CCK_{33} in the presence of memantine. [^{125}I]CCK_{33} (50 pM) was incubated with mouse cortex or striatum particles in the absence and presence of memantine. Degraded [^{125}I]CCK_{33} in the medium after 45 min of incubation was measured using the TCA precipitation method. Each value is given as per cent of the mean degraded material of two individual experiments run as duplicates.

	Degradation of tracer (%)
Mouse cortex	
None	5·20
Memantine (1 mM)	5·70
Memantine (10 mM)	5·55
Mouse striatum	
None	6·80
Memantine (1 mM)	6·85
Memantine (10 mM)	6·85

Figs 5 and 6 show the effect of memantine on the binding inhibition of labelled CCK by unlabelled CCK_8 in mouse cortex and striatum. Unlabelled CCK_8 competes for binding of [^{125}I]CCK_{33} in a concentration-related manner. In both mouse cortex and striatum 10 mM memantine increased the specific binding by 50 to 60% (Figs 5, 6). However, in both cases unlabelled CCK_8 was still able to compete for the binding of radiolabelled CCK with unchanged IC50 values.

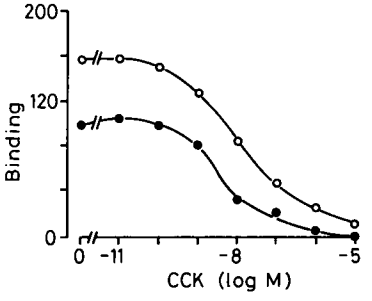

FIG. 5. Effect of memantine on [^{125}I]CCK_{33} binding to mouse cortex. [^{125}I]CCK_{33} (50 pM) was incubated with mouse cortex particles in the presence of increasing concentrations of unlabelled CCK_8 with (○) or without (●) 10 mM memantine. Values are given as per cent maximally bound in the absence of unlabelled CCK_8 and are the mean of three individual experiments run as duplicates.

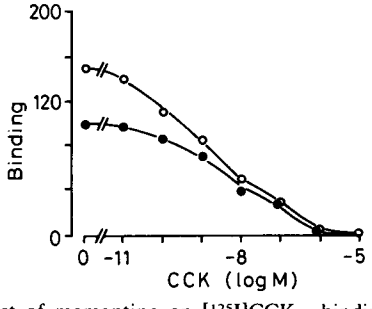

FIG. 6. Effect of memantine on [^{125}I]CCK_{33} binding to mouse striatum. [^{125}I]CCK_{33} (50 pM) was incubated with mouse striatum particles in the presence of increasing concentrations of unlabelled CCK_8 with (○) or without (●) 10 mM memantine. Values are given as per cent maximally bound in the absence of unlabelled CCK_8 and are the mean of three individual experiments run as duplicates.

Fig. 7 shows the effect of CCK and memantine on binding of labelled CCK to membranes of mouse acini. Unlabelled CCK_8 competed for binding of $[^{125}I]CCK_{33}$ in a concentration-related manner. Memantine, even at high concentrations, was without effect.

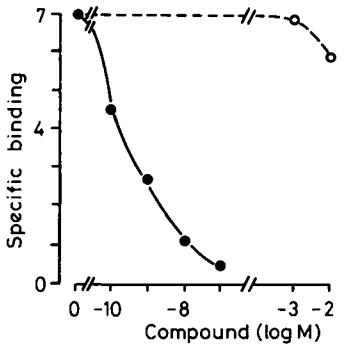

FIG. 7. Effect of unlabelled CCK_8 and memantine on specific $[^{125}I]CCK_{33}$ binding to mouse acinar membranes. $[^{125}I]CCK_{33}$ (50 pM) was incubated for 45 min with mouse acinar membranes plus increasing concentrations of (●) unlabelled CCK_8 or (○) memantine. Values are given as per cent maximally bound in the absence of unlabelled CCK_8 and are the mean of two individual experiments run as duplicates.

Discussion

The present study was designed to evaluate the interaction of memantine with brain CCK receptors. Our results together with those from previous studies (Gaudreau et al 1983; Hays et al 1980; Innis & Snyder 1980; Praissman et al 1983; Saito et al 1980; Saito et al 1981a, b; Van Dijk et al 1984; Zarbin et al 1983) demonstrate the presence of high affinity CCK receptors in membrane particles prepared from the cerebral cortex and striatum of mice. Our data on displacement of labelled CCK by unlabelled CCK are similar to the IC50 observed by others (IC50 = 1·3 nM; Steigerwalt & Williams 1984).

There was an increase in CCK binding by memantine in each experiment when mouse cortex and striatum were used. This effect of memantine on CCK binding in mouse brain is unique for two reasons: first, memantine has no effect on CCK binding to acinar membranes (Fig. 7), and second, memantine does not interact with the binding of other neurotransmitters to their receptors in rat brain membranes except 5-HT_1 (IC50 = 10 μM) as was shown by Osborne et al (1982).

The increase in binding observed in this study could be due to an indirect effect of memantine, e.g. due to inhibition of degradation of labelled CCK. This, however, was not the case since the degradation was small and not affected by memantine in either mouse cortex or striatum.

The observed effect of memantine on CCK binding is selective in that memantine does not affect [^{3}H]spiroperidol binding in striatal and frontal cortex, [^{3}H]dihydromorphine binding in striatum, [^{3}H]flunitrazepam and [^{3}H]muscimol binding in whole brain membranes, [^{3}H]clonidine and [^{3}H]WB 1401 binding in frontal cortex (Osborne et al 1982). However, memantine does exhibit a low affinity for [^{3}H]5-HT binding sites with an IC50 of 0·01 mM; it competed with 5-HT (Osborne et al 1982). Influence on receptor binding, however, was not tested for peptide ligands other than CCK. The increase in CCK binding observed in our studies is unique.

It is not clear whether our in-vitro data are relevant to in-vivo conditions. After i.p. injection of 100 μmol memantine kg^{-1} rat approx. 110 μmol drug kg^{-1} brain was measured (Wesemann et al 1982). This level is in the range of the IC50 of the memantine effect investigated in the present in-vitro system; however, the injected dose in this experiment was very high. In other in-vitro models memantine also is effective at rather the same concentration as was used in our experiments: release of 5-HT and dopamine from nerve endings of rat brain (0·5–5 mM) and release of 5-HT from blood platelets as a model of nerve endings (2 mM) (Haacke et al 1977). The concentrations used in our experiments are similar to those used by others in in-vitro investigations on memantine (0·01–0·25 mM; Wesemann et al 1982).

Whereas CCK effects on dopamine system are intensively investigated (for review see Crawley 1985) there are no biological data yet corresponding to the modulatory effect of dopamine or memantine on CCK and its receptors. This role has now to be evaluated.

Acknowledgements

We thank Dr J. A. Williams (Departments of Physiology and Medicine, University of California, San Francisco, USA) for the iodinated CCK_{33} and Dr G. Hallden (Karolinska Institute, Stockholm, Sweden, on leave in San Francisco) for conducting the experiments regarding the effect of memantine on CCK binding to plasma membranes of pancreatic acini.

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