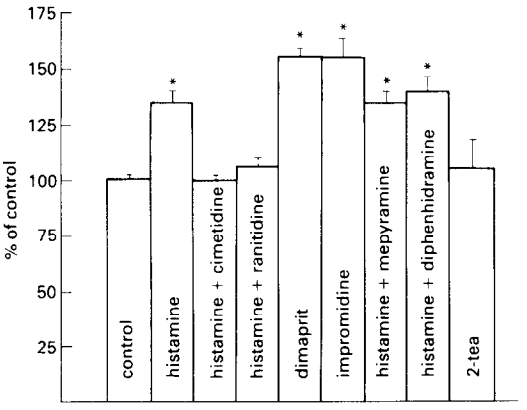

Fig. 3. Effect of HA antagonists and agonists on $^{45}Ca^{2+}$ uptake by rat brain synaptosomes. Uptake was measured at 1 min in R–T medium as described in Methods. Histamine 10^{-4} M: cimetidine 10^{-5} M; ranitidine 10^{-6} M; mepyramine and diphenhidramine 10^{-7} M; impromidine 5×10^{-5} M; dimaprit 10^{-4} M; 2-thiazoliletilamine (2-tea) 10^{-4} M. Data are expressed as percentage of controls. The control value for Ca^{2+} uptake was 5.526 ± 0.05. Results are means ± SEM; N = 5. (*) $P < 0.001$ vs control; Scheffe's test.

pletely blocked the effect of 10^{-4} M HA. In contrast, H_1 receptor antagonists, mepyramine and diphenhidramine, at 10^{-7} M did not alter the response induced by HA. In no case did either H_1 or H_2 receptor antagonists alone affect control $^{45}Ca^{2+}$ uptake levels (data not shown).

Dimaprit and Impromidine, selective H_2 receptor agonists at 10^{-4} M and 5×10^{-5} M, respectively, mimicked the effect of HA, whereas the H_1 receptor agonist 2-thiazoliletialmine (2-TEA) at 10^{-4} M was ineffective in modifying $^{45}Ca^{2+}$ uptake into synaptosomes with respect to controls. These results strongly suggest that HA-induced uptake of calcium into synaptosomes is mediated by H_2 receptors.

As already stated in the Introduction, the necessity of calcium ions for the process of NT release is well documented. Consistent with this generalized assumption, it has been recently reported that HA-induced release of dopamine from rabbit caudate nucleus slices as well as of acetylcholine in the guinea-pig ileum both depend critically on the extracellular Ca^{2+} concentration [12, 16]. Moreover, according to Pilc *et al.* [13], HA influence on the serotonergic system is mediated by HA H_2 receptors. The same receptor has been reported to be involved in the HA-induced release of dopamine in mammalian brain slices [12].

As both the release of the mentioned NT and the uptake of Ca^{2+} in response to HA seem to be mediated by H_2 receptors, it is plausible that these processes are related in some way. Therefore, we suggest that HA-induced entry of Ca^{2+} into nerve terminals could constitute an essential step for triggering the release of catecholamines or serotonin. Work is in progress to test this hypothesis.

In summary, our preliminary data indicate that HA induces an increased uptake of $^{45}Ca^{2+}$ into rat brain synaptosomes in a dose-dependent manner. This effect seems to be mediated by H_2 receptors. These findings could be related to a mechanism of control of HA-dependent NT release.

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Cyclic AMP efflux from rat striatal slices is enhanced by CCK

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Cholecystokinin (CCK) is one of several peptides which have been localised to neuronal elements of the basal ganglia of various species [1] and histological evidence has demonstrated that CCK and dopamine (DA) are co-localised in a large proportion of neurons in the mesolimbic system and medial substantia nigra [2–4], raising the possibility that CCK may modulate dopaminergic function.

CCK and dopamine have been shown to interact in a

number of studies [5–15]. This study set out to try and determine whether CCK affects dopaminergic transmission through an action on presynaptic or postsynaptic elements. There are two main sub-types of DA receptor in the CNS. Both D1 and D2 DA receptor sub-types occur in the rat striatum and affect the synthesis of adenosine 3′,5′ monophosphate (cyclic AMP). Stimulation of the D1 receptor increases cyclic AMP formation [16], while stimulation of the D2 receptor decreases the cyclic AMP formation stimulated by D1 agonists [17]. The experimental model we chose was an *in vitro* slice superfusion system similar to the one described by Stoof and Kebabian [17]. This system permits the concurrent study of possible interactions with D1 and D2 receptors. In addition, we used striatal homogenates to investigate possible direct interactions between CCK and adenylate cyclase-linked DA receptors.

Materials and methods

Adenylate cyclase activity. Rats were sacrificed by cervical dislocation and excised brain tissue was homogenised with a hand-held glass homogeniser in 5 vol. buffer containing 0.25 M sucrose, 5 mM $MgCl_2$, 25 mM KCl, 2 mM EGTA, 8 mM theophylline and 50 mM Tris–HCl, pH 7.4. The homogenates were centrifuged for 10 min at 1600 *g* and 4°. The supernatant was discarded and pellets resuspended in 20 vol. of the homogenising medium. The standard assay system contained in a final volume of 100 μl, 2 mM ATP, 10 μM GTP, 3 mM $MgCl_2$, 10 mM NaCl, 10 mM KCl, 1 mM EGTA, 8 mM theophylline in 50 mM Tris–HCl pH 7.4, either with/without test compounds. Bovine serum albumen (1 mg/ml) and bacitracin (100 μg/ml) were included where peptides were present. The reaction was started by the addition of 25 μl homogenate and incubated at 30° in a shaking water bath (120 strokes/min) for 15 min. Enzyme activity was terminated by boiling for 3 min. Samples of the supernatant were assayed for cyclic AMP using a protein binding saturation assay [18]. Protein was determined using the Lowry method [19].

Cyclic AMP release from striatal slices. Rat striatal tissue was chopped by passing it twice through a McIlwain tissue chopper (micrometer setting 300 μm) and the resulting blocks of tissue were transferred to well-oxygenated Earle's Balanced Salt Solution (EBSS), containing 116 mM NaCl, 5 mM KCl, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, 0.6 mM $MgSO_4$, 5.6 mM D-glucose and 1.3 mM $CaCl_2$, pH 7.4. The slices were mounted in Perspex chambers (30–40 mg tissue/chamber) and superfused for an hour at 0.1 ml/min. with EBSS fortified with 1 mM 3-isobutyl-1-methylxanthine (IBMX) and 2.5 mg/ml BSA, before the introduction of drugs. Bacitracin (200 μg/ml) was included in experiments using peptides. Cyclic AMP in 1 ml fractions of superfusate was determined using a radioimmunoassay (detection limit 2.5 fmol/100 μl; New England Nuclear). Basal cyclic AMP was determined over a 30 min period after the initial 60 min superfusion. Subsequently, slices were exposed to drug for 10 min and the next 10 min fraction collected for analysis of cyclic AMP. The amount of cyclic AMP formed in the presence of drug was expressed as a percentage of the basal cyclic AMP efflux, thereby correcting for differences in amounts of tissue present in each of the superfusion chambers. One superfusion channel in each experiment was left drug-free and drug treatments were randomised amongst the chambers to serve as additional controls.

Drugs. Test drugs were made up in experimental buffer after initial solvation in 4 mM tartarate or trace ascorbate (for DA agonists and antagonists), saline pH 4 (vasoactive intestinal peptide; VIP), 100 mM $NaHCO_3$ (sulphated CCK8), or absolute ethanol (forskolin).

Results

The effect of CCK on basal and dopamine stimulated adenylate cyclase activity in tissue homogenates. DA stimulates adenylate cyclase in a dose-dependent manner in all brain regions tested (nucleus accumbens, olfactory tubercle, amygdala, striatum, frontal cortex). However, sulphated CCK8 (CCK8S) at 10^{-5} M was without consistent effect either on its own or in the presence of DA (5–40 μM). The results shown in Fig. 1 show the dose-dependent activation of adenylate cyclase by DA and the DA agonist, SKF38393 (D1 receptor selective), forskolin and the peptide, VIP. CCK8S tested at a variety of concentrations was inactive. Furthermore, incubation of striatal membranes in the presence of forskolin at 10^{-9} and 10^{-4} M did not potentiate any possible stimulatory or inhibitory effect of CCK8S (results not shown).

Effect of CCK on clyclic AMP efflux from striatal slices. Both VIP and forskolin, which are known to activate adenylate cyclase, caused a significant increase in the efflux of cyclic AMP from striatal slices (Table 1). Sulphated CCK8 added at several concentrations (3×10^{-8}–10^{-5} M) also significantly enhanced cyclic AMP efflux, as shown in Fig. 2. The peptide increased release significantly at all concentrations tested, although a dose-related relationship was not obvious.

Effect of DA antagonists on CCK stimulated cyclic AMP efflux. SCH23390 (at 10^{-8} M) abolished the stimulation of efflux initiated by the addition of CCK8S (10^{-6} M) but was without effect on either VIP- or forskolin-stimulated efflux (Table 1). Sulpiride was also inhibitory on the response to CCK8S (Fig. 3). Figure 3 shows that this inhibition was stereoselective, (−)sulpiride being more potent than (+)sulpiride. Similarly, the effect was specific, VIP- and forskolin-stimulated efflux being unaffected by the addition of 10^{-6} M (−)sulpiride (results not shown).

Discussion

The ability of the peptides VIP and CCK and the diterpene, forskolin to stimulate cyclic AMP efflux from striatal slices has been demonstrated. VIP, CCK8S and forskolin

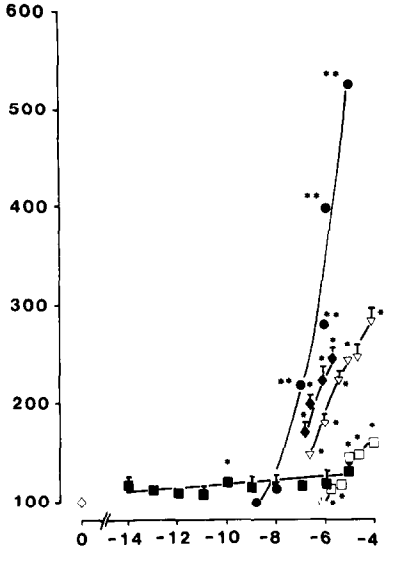

Fig. 1. Striatal homogenates were incubated for 15 min at 30° in the presence of ATP-containing buffer and drugs. The reaction was terminated by boiling and cyclic AMP assayed in the supernatant. Adenylate cyclase activity is expressed as % basal activity +1 SEM. Results are from 3–6 experiments assayed in triplicate: ▽, SKF38393; ◆, VIP; □, dopamine; ●, forskolin; ■, sulphated CCK8; *$P < 0.05$; **$P < 0.01$ Student's *t*-test; ordinate, %basal adenylate cyclase activity; abscissa, log. drug concentration (M). Basal adenylate cyclase activity was 45 ± 1 pmol cyclic AMP produced/min/mg protein.

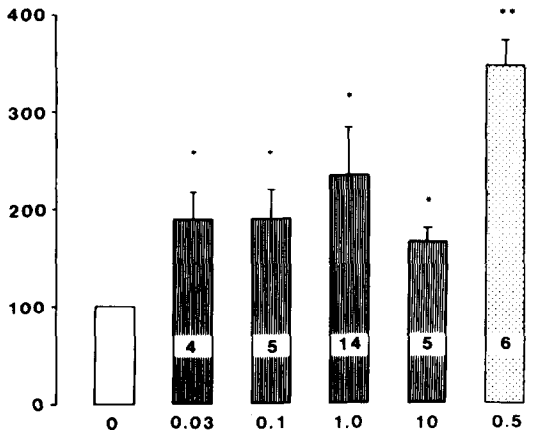

Fig. 2. Striatal slices were superfused with Earle's Balanced Salt Solution and cyclic AMP in the effluent assayed by radioimmunoassay. Tissue was in contact with drugs for 10 min and the subsequent 10 min fraction collected for assay of cyclic AMP as described in Methods. Results are expressed as a percentage of the cyclic AMP released from the slices in the 30 min period immediately prior to drug introduction (i.e. basal) and are mean + 1 SEM. The number of experiments is shown in each histogram. Basal efflux of cyclic AMP was 2.6 ± 0.17 fmol/mg tissue per 10 min: ▥, CCK8S; ▩, VIP; *P < 0.05; **P < 0.01 Student's *t*-test; ordinate, % basal efflux cyclic AMP; abscissa, drug concentration (μM).

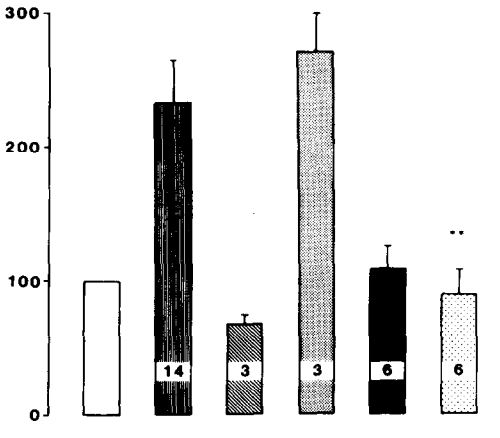

Fig. 3. Effect of stereoisomers of sulpiride on CCK8S-stimulated cyclic AMP efflux from rat striatal slices. CCK8S and sulpiride (both 10^{-6} M) were added to the superfusion medium together. Results are expressed as % basal efflux of cyclic AMP + 1 SEM. The number of experiments is given in the relevant histogram. **P < 0.01 relative to CCK8S alone by Student's *t*-test: □, Basal; ▥, CCK8S; ▧, (+)sulpiride; ▩, CCK8S plus (+)sulpiride; ■, (−)sulpiride; ▩, CCK8S plus (−)sulpiride ordinate, % basal efflux cyclic AMP.

all significantly stimulated efflux. Only the effects of CCK8S were influenced by DA antagonists. (−)Sulpiride (the active enantiomer for D2 receptor antagonism [20]), and SCH23390 (DI receptor antagonist [21]) both totally antagonised the effect of CCK8S whilst efflux of cyclic AMP induced by forskolin and VIP was unaffected. Our results thus suggest that CCK8S increases striatal efflux of cyclic AMP by a mechanism which is at least in part DA dependent. The exact mechanism by which the peptide exerts this effect appears to be complex.

CCK8S may be working via a presynaptic modulatory role, enhancing DA release. It would be anticipated that sulpiride might inhibit such a modulation by CCK8S whilst SCH23390 would inhibit the action of the released DA on the cyclase-linked receptor.

In any case, activation of the CCK receptor does not seem to be directly coupled to activation of adenylate cyclase, CCK8S having no effect on adenylate cyclase activity determined in tissue homogenates. Further, previous binding studies have failed to demonstrate any direct affinity to CCK8S for DA receptors [14].

Thus it is possible that CCK may be exerting an effect on DA transmission by binding close to the DA receptor or by influencing other mechanisms such as calcium influx or even nucleotide transport mechanisms. Further studies are needed to elucidate the nature of this considerable effect of CCK on the efflux of cyclic AMP, and to determine to how great an extent observed effects depend on the integrity of the dopaminergic system.

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Table 1. Effect of SCH23390 on cyclic AMP efflux from rat striatal slices

Drug Treatment	% Basal efflux	Significance from basal
Basal	100	
SCH23390 10^{-8} M	95 ± 10 (9)	NS
VIP 5×10^{-7} M	335 ± 38 (6)	**
VIP 5×10^{-7} M + SCH23390 10^{-8} M	353 ± 47 (6)	**
Forskolin 10^{-4} M	670 ± 320 (3)	**
Forskolin 10^{-4} M + SCH23390 10^{-8} M	480 ± 100 (3)	**
CCK8S 10^{-6} M	232 ± 38 (14)	*
CCK8S 10^{-6} M + SCH23390 10^{-8} M	92 ± 14 (8)	NS

Striatal slices were superfused with Earle's Balanced Salt Solution (EBSS) and cyclic AMP in the effluent assayed by radioimmunoassay using a specific antibody as described in Methods. The D1 dopamine receptor antagonist, SCH23390 (10^{-8} M) was added in combination with VIP, forskolin and CCK8S. The results are expressed as % basal efflux of cyclic AMP ± SEM with number of experiments in parentheses: *P < 0.05; **P < 0.01; NS = non significant relative to basal by Student's *t*-test.

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Hippocampal glutamate decarboxylase activity is not altered in gerbils with high seizure susceptibility

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Among different genetic animal models of epilepsy, i.e. genetically predisposed animal species in which seizures either occur spontaneously or in response to sensory stimulation, epilepsy-prone Mongolian gerbils exhibit unique features which make this species particularly useful for epilepsy research (cf. [1]). First, motor seizures can be reliably initiated in seizure-sensitive gerbils by simple external stimuli, such as change in environment [2], handling [3] or exposition of the animals to a blast of compressed air [4]. By the latter technique, seizures can be evoked in more than 98% of randomly bred gerbils, so that no selective breeding is necessary to obtain enough seizure-sensitive animals for experimental studies [5]. Second, the motor seizures are associated with epilepsy-like activity in the electroencephalogram, thus allowing evaluation of electrographic seizure phenomena [6]. Third, there is a progressive age-dependent development of seizure severity in gerbils [2, 5], which represents an interesting parallelity to human absence or myoclonic epilepsies in childhood, which at a later age often proceed to generalized tonic–clonic seizures. Fourth, antiepileptic drug efficacy studies in epileptic gerbils have shown that gerbils of different age can be used for identifying different clinical categories of antiepileptic drugs [5, 7].

The primary mechanisms underlying the seizure-proneness of gerbils are still unknown, although several recent studies have indicated that the inhibitory neurotransmitter γ-aminobutyric acid (GABA) may be involved [8–13]. Of special interest in this respect are recent experiments by Peterson *et al.* [12, 13] using immunocytochemical localization of the GABA-synthesizing enzyme glutamate decarboxylase (GAD), which have shown an increased number of GABAergic neurons in all regions of the hippocampus of seizure-sensitive gerbils compared to seizure-resistant ones. The most pronounced (up to 65%) increases in the number of GAD positive cells were found within the dentate gyrus and the CA 2,3 region of the hippocampal formation. In contrast to the findings in the hippocampal formation, no differences between seizure-sensitive and seizure-resistant gerbils were found in motor cortex, substantia nigra and nucleus reticularis thalami. The increased density of hippocampal GABAergic neurons in seizure-sensitive gerbils was thought to relate, at least in part, to an increased number of basket cells, which provide the main source of feedback and feedforward inhibition to the dentate gyrus and hippocampus proper [12, 13]. If these inhibitory neurons are acting to inhibit each other, the net effect of increased GABAergic activity would be disinhibition of the granule cells and pyramidal cells, which could lead to seizure activity within the hippocampal formation and at distant sites through multisynaptic connections [12, 13]. In fact, morphological studies in seizure-prone gerbils have indicated an increased activity of granule cells in the dentate gyrus, which could be explained by disinhibition [12–14]. The goal of the present paper was (1) to examine if the increase of hippocampal GAD activity found by Peterson *et al.* [12, 13] in seizure-susceptible gerbils by means of immunocytochemical methods can be confirmed when a technique for determination of GAD in synaptosomal fractions of discrete brain regions [15, 16] is used to assess nerve terminal activity of this enzyme, (2) to study if gerbils with different seizure-susceptibility differ in their hippocampal GAD activity, and (3) to determine synaptosomal GAD activities also in various other brain regions of seizure-susceptible and seizure-resistant gerbils. The results obtained do not substantiate the suggestion of Peterson *et al.* [12, 13] that in gerbils the hippocampus is a specific site for seizure-related differences in the GABAergic system.

Materials and methods

From the age of 7–8 weeks, randomly bred gerbils of both sexes were tested once a week for seizure-sensitivity and intensity of seizures. The testing procedure involved