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THE EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE ON RAT STRIATAL ^{3}H SPIPERONE BINDING

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Abstract—1. In the rat striatum sulphated CCK8 has no significant effect on equilibrium binding of ^{3}H-spiperone but has a considerable, although transient, effect under non-equilibrium conditions. 2. Under non-equilibrium conditions (during the association phase of ligand binding) and at high ligand concentrations (1 nM), CCK8 displaces specific binding and at low ligand concentrations (0.1 nM) CCK8 enhances specific binding. 3. CCK8 has no effect on ^{3}H-spiperone dissociation kinetics.

There is strong immunohistochemical evidence to suggest that in some neurones in the CNS cholecystokinin (CCK) and dopamine co-exist, and that the loss of dopamine function leads to CCK receptor supersensitivity in limbic areas [1–3]. Furthermore dopamine appears to both enhance and inhibit CCK release from the striatum; low concentrations enhancing and high concentrations inhibiting [4].

The effects of CCK on markers of dopaminergic functions are less clear. For instance there are reports that suggest the peptide will enhance dopamine turnover [5] and stimulate potassium-induced striatal dopamine release [6]. However, many of the reports concerned with the effect of CCK on DA activity measured by ligand binding assays under equilibrium conditions are contradictory. Thus CCK is said to increase the affinity and reduce the number of binding sites using ^{3}H-spiperone [7, 8] or ^{3}H-dopamine [9] and to decrease the number of binding sites when the ligand is ^{3}H-*n*-propylnorapomorphine whilst having no effect on the affinity of the receptors for the ligand [10].

In the light of these discrepancies we have looked more closely at the kinetics of ^{3}H-spiperone binding in the absence and presence of CCK.

MATERIALS AND METHODS

Striatae from female 250 g Wistar rats were homogenised in 50 volumes of 50 mM Tris (hydroxymethyl) amino methane (Tris–HCl) buffer pH 7.7. Membranes were collected by centrifugation at 50,000 *g* for 10 min and washed twice with 50 volumes Tris–HCl buffer. After the first washing the membrane suspension was preincubated for 10 min at 37° to remove endogenous catecholamines. The final membrane pellet was resuspended in 100 volumes of 50 mM Tris–HCl buffer (pH 7.4) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 50 μM pargyline and 0.1% ascorbic acid. Bovine serum albumin (1 mg/ml) and bacitracin (100 μg/ml) were included in all of the experimental tubes. ^{3}H-spiperone (specific activity 25.7 Ci/mmole; NEN, Germany) was used at a final concentration of 0.1–1 nM for kinetic studies and 0.1–2 nM for equilibrium studies. ^{3}H-ligand binding was performed in a volume of 1 ml composed of 850 μl membrane suspension, 50 μl ^{3}H-ligand, 50 μl peptide or solvent, 50 μl spiperone, (+) butaclamol or solvent.

Binding was carried out for 20 minutes at 25° (for equilibrium studies). Bound radioactivity was collected by filtration through Whatman GF/B filters. Filters were washed with 2 × 10 ml 50 mM Tris–HCl buffer and bound radioactivity estimated in an Intertechnique SL 4000 liquid scintillation counter, corrected for background, quenching, machine efficiency and filter blanks.

Sulphated CCK-8 (from I.C.I.) was dissolved in a small amount of 100 mM sodium bicarbonate before further dilution with Tris–HCl buffer (pH 7.4).

Association rate studies. 850 μl of the membrane suspension was added to tubes containing 50 μl of 10% ethanol or 2 μM (+) butaclamol in 10% ethanol, and 50 μl CCK-8 (10^{-13}–10^{-5} M) or solvent. The tubes were incubated for 20 min before the addition of ^{3}H-spiperone. The reaction was terminated by filtration at times varying from 0 to 60 min. Association binding data was fitted to a pseudo first order rate equation where B_{eq} = the maximum specific binding at equilibrium and B_t = the amount of label specifically bound at time t. Pet Commodore computer assisted linear regression was used to obtain a value for k_{obs}.

Dissociation rate studies. Membranes were preincubated at 25° for 20 min ± CCK-8 (10^{-13}–10^{-5} M). ^{3}H-spiperone was then added and allowed to reach an equilibrium binding state (25 min at 25°). Two μM (+) butaclamol or 1 μM cold spiperone were then added to initiate dissociation of the receptor-bound ^{3}H-spiperone. The reaction was terminated by rapid filtration after 0–60 min. Dissociation binding data were fitted to a first order rate equation. The negative slope was used to directly estimate k_{-1}, the dissociation rate constant.

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Table 1. ^{3}H-spiperone binding (0.1–2 nM): effect of CCK-8 on receptor number and affinity

Drug treatment	K_d (nM)	B_{max} (fmoles/mg protein)	Number of experiments
Control	0.106 ± 0.025	115 ± 7	6
1 μM CCK-8	0.099 ± 0.02	112 ± 6	3
10 μM CCK-8	0.078 ± 0.015	104 ± 7	3

RESULTS

The effect of CCK8 at two concentrations (1 and 10 μM) on the equilibrium dissociation constant (K_d) and maximal binding capacity (B_{max}) of ^{3}H spiperone binding to rat striatal membranes is shown in Table 1. In contrast to previously reported data [8] the peptide had no significant effect on either of these parameters.

Furthermore, CCK8 (10^{-10}–10^{-5} M) did not displace ^{3}H-spiperone binding at equilibrium, even after prolonged preincubation (up to 60 min) in the presence of bacitracin and BSA.

Although it is possible that the variable effects of CCK seen by others could be a reflection of variations in endogenous dopamine levels in the preparations used in the various studies, this explanation seemed unlikely since CCK (0.1–20 μM) had no effect on the IC_{50} for (+)-butaclamol (IC_{50} = 20 ± 0.1 nM) or dopamine (IC_{50} 10 ± 0.2 μM) nor did it affect (+) butaclamol displacement after preincubation with low concentrations of dopamine (10 nM).

Another potential source of the disparent results in the literature is that the peptide may be modulating the rate of association and/or rate of dissociation of spiperone from its binding site in a differential fashion. Such an effect would make the timing of equilibrium studies crucial and could account for different observations with different experimental protocols. This possibility was investigated by examining the effect of CCK (10^{-5} M) on the association rate of specific ^{3}H spiperone binding to rat striatal membranes and results are shown in Fig. 1. Clearly, in the presence of CCK the rate of association is significantly reduced over the first 15 min of the experiment when compared with control values in the absence of CCK. In contrast none of the concentrations of CCK 8 tested (10^{-13}–10^{-5} M) was found to affect the dissociation rate when either 2 μM (+) butaclamol or 1 μM spiperone was used to initiate dissociation of the bound ligand (using 0.1–1 nM ^{3}H-spiperone). The association and dissociation rate constants in the presence and absence of 10^{-5} M CCK are shown in Table 2. The results in Table 2 show that this concentration of CCK significantly increases the equilibrium dissociation constant. However, if the K_{obs} is calculated from the data collected over a 10-min period the equilibrium dissociation constant is not significantly altered by the presence of CCK (K_d 0.092 ± 0.001 nM in absence and 0.098 ± 0.006 nM in presence of peptide).

Further studies in which different concentrations of ^{3}H-spiperone were tested in the absence and presence of sulphated CCK-8 (10^{-13}–10^{-5} M) and with incubation times of only 8 min (i.e. studies carried out in the association phase of ligand binding) revealed an interesting effect. At high concentrations of ^{3}H-spiperone (1 nM) CCK-8 produced a dose-dependent displacement of specific ligand binding and at low ligand concentrations (0.1 nM), CCK-8 enhanced specific binding (Table 3).

Table 3. Effect of sulphated CCK-8 on specific ^{3}H-spiperone binding: a non-equilibrium situation

CCK-8 (M)	% Change in specific ^{3}H-spiperone binding using 2 ligand concentrations A	B
0	0	0
10^{-13}	+80	−20
10^{-11}	+84	−20
10^{-9}	+60	−40
10^{-7}	+65	−45
10^{-5}	+85	−60

Membranes were incubated for 20 min at 25° in the absence or presence of CCK-8, then for a further 8 min in the presence of ^{3}H-spiperone (A = 0.1 nM ligand, B = 1 nM ligand). Binding was terminated by rapid vacuum filtration. For 2 separate experiments using the same concentration of membrane protein and the same ligand concentrations, results varied by less than 10%.

Table 2. Rate constants for ^{3}H-spiperone: effect of CCK-8 at 10 μM

Drug treatment	K_{obs} (min^{-1})	K_{-1}	K_{eq} (nM)
Control	0.103 ± 0.007 (9)	−0.021 ± 0.002 (9)	0.056 ± 0.003
+CCK-8 (10 μM)	0.069 ± 0.008 (3)*	−0.019 ± 0.004 (3) N.S.	0.071 ± 0.007†

Number of experiments in parenthesis.
N.S. not significantly different from control.
* Significantly different from control ($P < 0.01$).
† Significantly different from control ($P < 0.05$) paired t-test.

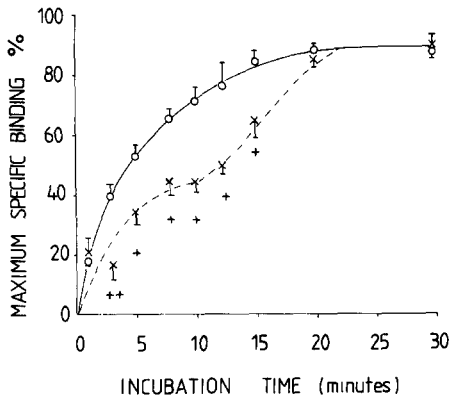

Fig. 1. Effect of CCK on the association rate of specific ^{3}H-spiperone binding to rat striatal membranes. ○, control; ×, plus 10 μM CCK 8. Each point is mean ± S.E.M. for control N = 9, +CCK N = 3. ++P < 0.01, +P < 0.05 when compared to control values using Student's *t*-test. Approximate ligand concentration used is 0.3 nM.

DISCUSSION

These results demonstrate that the effects of CCK-8 on ^{3}H-spiperone binding in the rat striatum are only seen in the non-equilibrium phase of binding experiments where the changes in specific binding in the presence of CCK-8 can be as large as 20–85%. These results do not reflect an effect on the dissociation kinetics measured at equilibrium. At equilibrium, the binding of ^{3}H-spiperone is not subjected to more than small modulatory effects of CCK8 which may be of the order of 10% [7, 8].

Thus our results suggest that the CCK octapeptide may be acting exclusively on the association rate kinetics, although it is possible that CCK may be influencing the maximal binding capacity in a transient manner (see Fig. 1). The transient nature of the response is similar to the reported effect of VIP in the rat submandibular salivary gland [11] on ^{3}H-*N*-methyl-4-pipiridinyl benzilate binding. This effect was maximal after 3–4 min and undetectable at 10 min. Again no effect of the peptide on equilibrium binding was observed.

The transient nature of both the above effects may reflect modulation by peptides (here CCK and VIP) of specific ligand binding which is dependent on the degree of receptor occupancy. However, whether or not these changes in receptor occupancy/affinity represent an important way in which CCK might modulate dopaminergic transmission is unclear.

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