Subcortical limbic $^3$H-N-propynorapomorphine binding sites are markedly modulated by cholecystokinin-8 in vitro

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By means of the dopamine (DA) agonist radio ligand $^3$H-N-propynorapomorphine ($^3$H-NPA) the effects of cholecystokinin-8 (CCK-8) have been evaluated in vitro on the binding characteristics of the DA agonist sites in membrane preparations from the subcortical limbic forebrain containing mainly nucleus accumbens and tuberculum olfactorium. It was shown that CCK-8 (10$^{-8}$ M) can produce a 40% increase in the K$_D$ value of the $^3$H-NPA binding sites and a significant 10% increase in the B$_{max}$ values of these sites. It is therefore suggested that there exist marked receptor-receptor interactions between the CCK-8 binding sites and DA agonist binding sites in the limbic forebrain. On the basis of these findings and in view of the fact that CCK peptides are comodulators in certain types of mesolimbic DNA neurons but cannot modulate DA release in these DNA synapses, the hypothesis is introduced that the presence of DA comodulators such as CCK-8 in the DA synapses makes possible a heterostatic regulation of the synapse. Thus, by means of receptor-receptor interactions, peptide comodulators may change the set point of the main transmission line without inducing homeostatic feedback responses on synthesis and release of the main transmitter, opening up a new way to modulate chemical transmission in general.

Previous studies (Fuxe et al., 1981; Agnati et al., 1983a,b) have demonstrated that CCK peptides can produce a differential regulation of $^3$H-spiperone (a dopamine (DA) receptor antagonist radio ligand) and $^3$H-N-propynorapomorphine ($^3$H-NPA) (a DA agonist radio ligand) binding sites in striatal membranes. Thus, CCK-8 (10 nM) can in vitro increase the affinity and reduce the number of $^3$H-spiperone-labelled DA receptors in striatal membranes, while CCK-8 in the same concentration (10 nM) reduces the affinity and increases the number of $^3$H-NPA binding sites in striatal membranes. These results indicated that $^3$H-agonist- and $^3$H-antagonist-labelled sites of the
striatal DA receptors represent different entities and that one type of change induced by CCK-8 is one which resembles receptor interconversion, caused by the existence of CCK-DA receptor interactions in the same striatal local circuits (see Fuxe et al., 1983).

In the present paper we have investigated whether CCK-8 (10 nM) can more markedly modulate the ³H-agonist binding sites of the subcortical limbic than of the striatal DA receptors, since in limbic areas several of the DA terminals may co-store CCK-8 as a comodulator (Hökfelt et al., 1980). Therefore, an improved space locked interaction between CCK-8 and DA receptors may take place in the subcortical limbic forebrain (mainly nucleus accumbens and tuberculum olfactorium) compared with striatum. This investigation is of special interest also in view of the fact that CCK-8 given intraventricularly into awake animals does not modulate DA turnover in those limbic DA terminals which co-store CCK-like immunoreactivity but only in those DA terminal systems which codistribute with CCK immunoreactive terminals making axo-axonic contacts possible between the systems (Fuxe et al., 1980).

Materials and Methods

Male specific-pathogen-free Sprague-Dawley rats (150 g body wt.) were used. The rats were given food pellets and water ad libitum and kept under constant light and dark conditions (lights on at 0600 and off at 2000). After decapitation the limbic tissue was dissected out. It contained mainly nucleus accumbens and tuberculum olfactorium on the two sides and the basal forebrain area in between. As landmarks, tractus olfactorius lateralis and the anterior limb of the anterior commissure were used. The tissue was dissected out from the coronal section of the telencephalon (3 mm thick), the rostral border being located at the anterior end of the tuberculum olfactorium. The tissue was processed as fresh tissue. It was sonicated for 20 s at the maximal setting using a Prep 150 (MSE). The ³H-NPA (61.2 Ci/mmol, NEN, Boston, Massachusetts, USA) binding procedure was performed as described by Leysen & Gommeren (1981) for ³H-apomorphine binding. Unspecific binding was defined as binding in the presence of 10⁻⁶ M of (+) butaclamol. The tissue concentration was 10 mg/ml and the protein concentration was determined by the Lowry method. The limbic membranes were preincubated for 10 min at 37°C to remove endogenous DA and preincubation with CCK-8 (10 nM) was performed for 10 min at 4°C. The ³H-NPA binding took place at room temperature during a period of 30 min. CCK-8 (10 nM, Peninsula Laboratories, San Carlos, California, USA) was used in the presence of 10⁻⁶ M of bacitracin and 0.05% bovine serum albumin. The incubation was stopped by filtration using GF/B filters in a multiple filtration apparatus (Multividor, Janssen Pharmaceuticals, Beerse, Belgium). The membranes were washed 3 times with 5 ml of cold buffer. For liquid-scintillation counting a high-efficiency premixed cocktail for aqueous and nonaqueous samples was used (Ready Solv™ HT/d, 4 ml). Prior to adding ReadySolv™, 0.4 ml of 0.1 M KOH was added to the filters, and the filters were incubated in this solution for 30 min. Saturation analysis was performed to study
The effects of CCK-8 on the binding characteristics of $^3$H-N-propylnorapomorphine binding sites (8-10 concentrations). In each experiment the solvent group was run in parallel with the CCK-8 group. The correlation coefficient for the linear fitting in the Scatchard plot ranged from 0.85 to 0.99. 14 replications were made and a possible significant action on the $K_D$ (nM) and $B_{max}$ values (fmol/mg prot) was evaluated using Student's paired t-test.

Results

It is shown in Fig. 1 that CCK-8 (10 nM) can produce a marked and highly significant reduction in the affinity of the $^3$H-NPA binding sites in subcortical limbic membranes. The $K_D$ value was increased by approx. 40%, when compared with the solvent-treated-group mean value. A small but significant increase in the $B_{max}$ values of the $^3$H-NPA binding sites was also noted under the influence of CCK-8 (10 nM). This increase was of the order of 10% (Fig. 1). CCK-8 itself did not displace $^3$H-NPA from its binding sites in concentrations of $10^{-6}$ to $10^{-8}$ M.

Discussion

The present results demonstrate that the $^3$H-NPA binding sites in subcortical limbic membranes are more markedly affected by CCK-8 than those in striatal membranes, when considering the affinity changes induced by CCK-8. Thus, in striatal membranes a 20% increase in the $K_D$ value of the $^3$H-NPA binding site is observed in the presence of CCK-8 (10 nM) (Agnati et al., 1983) whereas in
subcortical limbic membranes the $K_D$ value is increased by 40%. In contrast to this the small but significant increase in the $B_{\text{max}}$ values is observed in the $^3$H-NPA binding sites in the presence of CCK-8 in both striatal and subcortical limbic membranes. These results indicate that the CCK-DA receptor interactions may be more pronounced in areas where DA-CCK coexistence occurs. Thus, at DA-CCK synapses more DA receptors may be linked to CCK receptors and/or the steric interaction between these two populations of receptors may be more pronounced.

The present results are in sharp contrast to the results obtained on the presynaptic DA side, since CCK-8 cannot modulate DA turnover in nerve terminals co-storing DA and CCK-like peptides (Fuxe et al., 1980). Thus, the DA comodulator CCK-8 seems to have the unique capacity of inducing a change in the decoding of the DA message at the postsynaptic cell-membrane level without inducing homeostatic responses in DA synthesis and release. Thus, by markedly changing the affinity of the $^3$H-NPA binding sites labelling the $D_2$ and $D_3$ subtypes of DA receptors, CCK-8 can change the set point of the feedback controlling the DA transmission. In fact, there is a change in the sensitivity of the decoding system without inducing at the same time homeostatic responses, which is always found following treatment with DA agonists or antagonists. Thus, the present results reveal the possibility that one function of coexistence of transmitters may be to provide a mechanism for the heterostatic regulation of chemical transmission. Thus, the set point for the DA (main) transmission line can be changed without triggering homeostatic mechanisms which tend to maintain the constancy of DA transmission. In this way we have a new way to modulate DA receptor activity and thus to develop new therapeutic drugs, which act on the peptide comodulator line in the DA synapses. In view of the involvement of limbic DA receptors in schizophrenia it seems possible that drugs, which can mimic CCK-8 or neurotensin, which also markedly reduces the affinity of DA receptors in the limbic system (Agnati et al., 1983c) may represent a new treatment of this mental disease. Thus, these neuropeptides may stabilize subcortical limbic DA transmission due to their ability to markedly reduce the affinity of the agonist binding sites of the DA receptor.

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References