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Modulation of motor behaviour by NMDA- and cholecystokinin-antagonism

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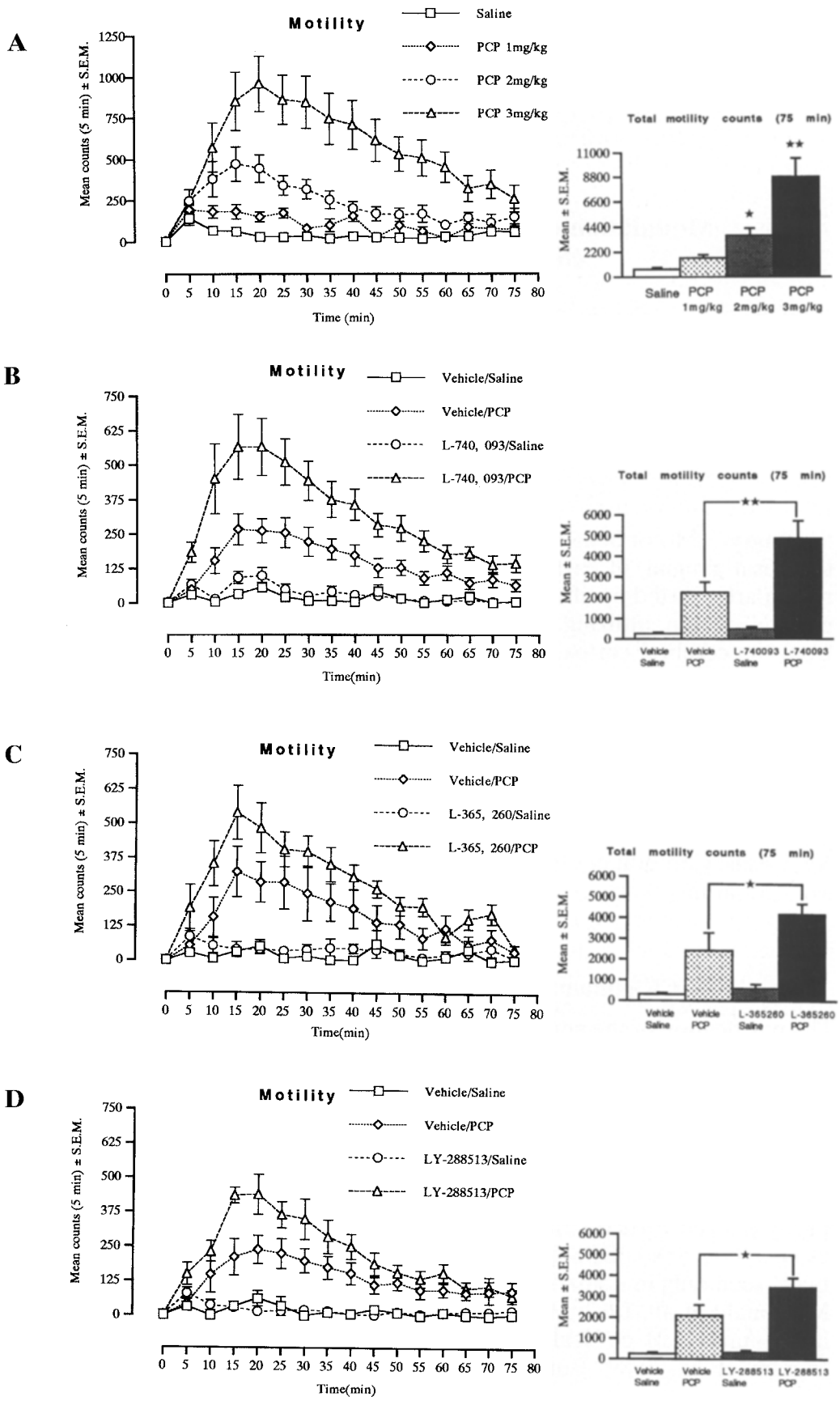
Summary. Motor behaviour relies on complex neurochemical interactions in the basal ganglia, in particular the striatum. Antagonistic influences in this region are exerted by afferent projections from, on the one hand, the ventral mesencephalon, utilizing dopamine as a transmitter, and, on the other hand, from the cerebral cortex, signalling by the excitatory amino acid glutamate. The activity in both these neuronal populations appears to be regulated by the neuropeptide cholecystokinin. This article concentrates on interactions between cholecystokinin and glutamate, summarizing some recent morphological, biochemical and behavioural findings. It is suggested that cholecystokinin, acting via the cholecystokinin$_B$ receptor, potentiates the glutamatergic excitatory input to the striatum.

Keywords: Cerebral cortex – Glutamate – Nucleus accumbens – Schizophrenia – Striatum

CCK-glutamate interactions: Morphological studies

The purification of the neuropeptide cholecystokinin (CCK) from gut extracts by Jorpes and Mutt in 1968 (Mutt and Jorpes, 1968) led to the discovery of high levels of a gastrin/CCK-like peptide also in the brain (Vanderhaeghen et al., 1975). CCK has been found to be present in many neuronal populations also containing "classical", low- molecular weight transmitters. Thus, CCK is co-localized with dopamine in neurones of the ventral mesencephalon projecting to forebrain regions (Hökfelt et al., 1980). Later, the existence of a major, partly crossed cortico-striatal CCK-ergic pathway was demonstrated (Morino et al., 1992, 1994). CCK-like immunoreactivity in the cerebral cortex is normally seen only in a small number of cell bodies (Vanderhaeghen et al., 1981; McDonald et al., 1982; Hendry et al., 1983; Peters et al., 1983). However, a large number of cortical cells express CCK mRNA (Savasta et al., 1988; Ingram et al., 1989; Burgunder and Young, 1990), suggesting that CCK


A
Motility
Saline
PCP 1mg/kg
PCP 2mg/kg
PCP 3mg/kg
Mean counts (5 min) ± S.E.M.
Time (min)
Total motility counts (75 min)
Mean ± S.E.M.
Saline
PCP 1mg/kg
PCP 2mg/kg
PCP 3mg/kg
B
Motility
Vehicle/Saline
Vehicle/PCP
L-740, 093/Saline
L-740, 093/PCP
Time(min)
Total motility counts (75 min)
Vehicle Saline
Vehicle PCP
L-740093 Saline
L-740093 PCP
C
Motility
Vehicle/Saline
Vehicle/PCP
L-365, 260/Saline
L-365, 260/PCP
Time(min)
Total motility counts (75 min)
L-365260 Saline
L-365260 PCP
D
Motility
Vehicle/Saline
Vehicle/PCP
LY-288513/Saline
LY-288513/PCP
Time(min)
Total motility counts (75 min)
LY-288513 Saline
LY-288513 PCP

produced in the cortex is rapidly transported to terminal ramifications. Morino et al. (1994) showed that a unilateral injection of the tracer wheat germ agglutinin in the striatum labelled cell bodies co-staining with pro-CCK antibodies in both cortical hemispheres. Following combined decortication and callosotomy, CCK-like immunoreactivity was strongly decreased in the ipsilateral striatum. While it is generally accepted that glutamate is the major transmitter of the corticostriatal projection (Kim et al., 1977; McGeer et al., 1977; Reubi and Cuenod, 1979; Fonnum et al., 1981; Fagg and Foster, 1983), it remains to be established whether or not CCK and glutamate actually coexist in this pathway. It has, however, been shown that these two neurochemicals are present in striatal terminals, in both cases with the ultra-morphological characteristics of corticostriatal afferents (Snyder et al., 1993).

Innis and Snyder (1980) first postulated the existence of two separate receptors for CCK, the CCK_A- and the CCK_B-receptors. The recent clonings of these receptors (Wank et al., 1992a; b) have made it possible to determine their expression patterns in the brain by in situ hybridization. While CCK_B receptor mRNA is found with a wide distribution, including the cerebral cortex, nucleus caudate-putamen and nucleus accumbens, CCK_A receptor mRNA is found only in a limited number of brain regions, including the hypothalamus and cerebral cortex (Honda et al., 1993; Broberger and Hökfelt, unpublished observations).

CCK-glutamate interactions: Biochemical studies

Under normal conditions, extracellular levels of CCK in the striatum are relatively low (Meana et al., 1991), but can be dramatically increased by perfusion with K^+, an effect that is Ca^{2+}-dependent, indicating a neuronal source for releasable CCK (You et al., 1994). Decortication combined with callosotomy significantly decreased basal, as well as K^+-induced, CCK-release in the striatum (Herrera-Marschitz et al., 1992), in agreement with the morphological studies cited above.

An interesting interaction between CCK- and *N*-methyl-D-Aspartate (NMDA)-receptors has been found in the striatum, where the major neuronal population, the striatal spiny projection neurones, synthesize a dopamine- and cAMP-regulated 32-kDa phosphoprotein (DARPP-32) (Ouimet et al., 1984). DARPP-32 is phosphorylated following activation of the dopamine D1 receptor (Hemmings et al., 1984), and dephosphorylated following activation of the NMDA receptor (Halpain et al., 1990). It has been demonstrated that, in a

Fig. 1. Effect of different doses of PCP (**A**) and of combined treatment of the CCKB antagonists L-740,093 (**B**), L-365,260 (**C**) and LY-288513 (**D**) and PCP on motility patterns in habituated male, adult, Sprague-Dawley rats. Compounds were administered in the following dosages: (**A**) PCP 1; 2; 3 mg/ kg s.c. (**B**) PCP 2 mg/kg s.c.; L-740,093 1 mg/ kg i.p. (**C**) PCP 2 mg/kg s.c.; L-365,260 10 mg/kg i.p. (**D**) PCP 2 mg/kg s.c.; LY-288513 10 mg/kg i.p. Note potentiation of PCP-induced hyperlocomotion induced by all three CCK_B-antagonists. $^*p < 0.05$, $^{**}p < 0.01$. For details, see Blacker et al., 1997

striatal slice preparation, CCK produces a decrease in the phosphorylation of DARPP-32 (Snyder et al., 1993). This effect was counteracted by simultaneous application of not only the CCK_B-antagonist CI-988, but also by the non-competitive NMDA-antagonist dizocilpine (MK-801). However, when CCK was given together with NMDA, no additional decrease of DARPP-32 phosphorylation could be seen, indicating that CCK may stimulate the release of glutamate, or activate aspartate interneurones shown to be present in the striatum (Snyder et al., 1993; Pettersson et al., 1996).

CCK-glutamate interactions: Behavioural studies

Thus, morphological and biochemical data indicate that CCK and glutamate may interact to influence motor behaviour. In a series of recent experiments (Blacker et al., 1997), we addressed the functional significance of this neurochemical substrate in the regulation of motor behaviour, studying the influence of CCK and glutamate antagonists on locomotion. The non-competitive NMDA receptor antagonist phencyclidine (PCP) (Fagg, 1987) has been particularly studied for its ability to induce a psychotomimetic state in humans resembling schizophrenia (for review, see Javitt and Zukin, 1991). In our study, PCP (2 mg/kg, s.c.) induced a marked increase in both the amplitude and duration of locomotion in habituated animals as compared to the baseline activity of saline-injected controls (see figure). When injection of PCP was preceded by 15 min of an i.p. injection of the CCK_B-antagonists L-365,260 (Parke-Davis; 10 mg/kg); LY-288513 (Eli Lilly; 10 mg/kg) or L-740,093 (Merck Research Laboratories; 1 mg/kg), there was an immediate and continuing potentiation of the PCP-induced hyperactive motor behaviour (see Fig. 1). Similar results were also obtained when L-740,093 was combined with MK-801 (0.15 mg/kg). However, neither of the CCK_B-antagonists have any significant effect on the baseline locomotion activity of habituated animals when administered alone. Thus, it may be speculated that glutamate antagonism induces endogenous release of CCK.

In a second series of experiments, CCK_B antagonists have been administered to non-habituated animals, which were immediately placed in locomotor boxes following drug administration. A significant reduction in exploratory behaviour was observed with L-365,260 (10 mg/kg) and LY-288513 (10 mg/kg). This is thus another example of endogenous release of CCK acting on CCK_B receptors, however with an opposite behavioural effect to that observed by PCP-treatment. Interestingly, it has been reported (Ladurelle et al., 1995) that when rats are in a familiar environment, injection of CCK-8S into the nucleus accumbens causes a short, transient release of dopamine, but when transferred to a novel environment, CCK-8S induces a marked prolongation of dopamine release, indicating an involvement of CCK in exploratory behaviour.

These results, in combination with the studies reviewed above, support a model where dopamine on the one hand, and glutamate and CCK_B receptor activation, on the other, balance each other in the regulation of motor behaviour. CCK has been shown to antagonize the effect of dopamine transmission at

several levels, including PCP-induced dopamine release (Kuroki et al., 1992). Blocking the NMDA-pathway by PCP or MK-801 will favour dopaminergic stimulation of locomotion. To counteract this, CCK may be released, possibly from glutamatergic nerve endings, to stimulate increased glutamate release via the CCK_B-receptor. This model would agree with the CCK/glutamate regulation of DARPP-32 shown by Snyder et al. (1993), although many questions concerning such an interaction remain to be addressed.

In conclusion, CCK is found in regions of the brain involved in the modulation of motor behaviour, co-localized, or in close association, with glutamate and dopamine. Endogenous CCK, of neuronal origin, can be released in the brain following NMDA receptor antagonism, and may act to modify behaviour induced by NMDA-blockade. The CCK/glutamate interaction may represent a novel target for the treatment of psychotic disorders.

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References

Blacker D, Broberger C, Ögren SO, Hökfelt T (1997) Cholecystokinin B receptor antagonists enhance the locomotor response to the *N*-methyl-D-aspartate antagonists phencylidine and dizocilpine maleate. Neuroscience 76: 1057–1067

Burgunder JM, Young WS III (1990) Cortical neurons expressing the cholecystokinin gene in the rat: distribution in the adult brain, ontogeny, and some of their projections. J Comp Neurol 300: 26–46

Fagg G, Foster AC (1983) Amino acid neurotransmitters and their pathways in the mammalian central nervous system. Neuroscience 9: 701–719

Fagg GE (1987) Phencyclidine and related drugs bind to the activated *N*-methyl-D-aspartate receptor-channel complex in rat brain membranes. Neurosci Lett 76: 221–227

Fonnum F, Storm-Mathisen J, Divac I (1981) Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibres in rat brain. Neuroscience 6: 863–873

Halpain S, Girault JA, Greengard P (1990) Activation of NMDA receptors induces dephosphorylation of DARPP-32 in rat striatal slices. Nature 343: 369–372

Hemmings HC Jr, Greengard P, Tung HYL,Cohen P (1984) DARPP-32, a dopamine-regulated neuronal phosphoprotein, is a potent inhibitor of protein phosphatase-1. Nature 310: 503–505

Hendry SHC, Jones EG, Beinfeld MC (1983) Cholecystokinin-immunoreactive neurons in rat and monkey cerebral cortex make symmetric synapses and have intimate associations with blood vessels. Proc Natl Acad Sci 80: 2400–2404

Herrera-Marschitz M, Meana JJ, Hokfelt T, You ZB, Morino P, Brodin E, Ungerstedt U (1992) Cholecystokinin is released from a crossed corticostriatal pathway. Neuroreport 3: 905–908

Honda T, Wada E, Battey JF, Wank SA (1993) Differential gene expression of CCK_A and CCK_B receptors in the rat brain. Mol Cell Neurosci 4: 143–154

Hökfelt T, Skirboll L, Rehfeld JF, Goldstein M, Markey K, Dann O (1980) A subpopulation of mesencephalic dopamine neurons projecting to limbic areas contains a chole-

cystokinin-like peptide: evidence from immunohistochemistry combined with retrograde tracing. Neuroscience 5: 2093–2124
Innis RB, Snyder SH (1980) Distinct cholecystokinin receptors in brain and pancreas. Proc Natl Acad Sci 77: 6917–6921
Ingram SM, Krause RG, Baldino IF, Skeen LC Jr, Lewis ME (1989) Neuronal localization of cholecystokinin mRNA in the rat brain by using in situ hybridization. J Comp Neurol 287: 260–272
Javitt DC, Zukin SR (1991) Recent advances in the phencyclidine model of schizophrenia. Am J Psychiat 148: 1301–1308
Kim JS, Hassler R, Haug P, Paik KS (1977) Effect of frontal cortex ablation on striatal glutamic acid level in rat. Brain Res 132: 370–374
Kuroki T, Takebayashi Y, Ide K, Yonezawa Y, Tsutsumi T, Matsumoto T, Hirano M, Uchimura H (1992) Antagonism of ceruletide, a cholecystokinin analog, to the neurochemical effects of the non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, phencyclidine and MK-801, on regional dopaminergic neurons in the rat brain. Neuropeptides 21: 167–173
Ladurelle N, Roques BP, Daugé V (1995) The transfer of rats from a familiar to a novel environment prolongs the increase of extracellular dopamine efflux induced by CCK8 in the posterior nucleus accumbens. J Neurosci 15: 3118–3127
McDonald JK, Parnavelas JG, Karamanlidis A, Brecha N, Rosenquist G (1982) The morphology and distribution of peptide-containing neurons in the adult and developing visual cortex of the rat. III. Cholecystokinin. Neurocytol 11: 881–895
McGeer PL, McGeer EG, Schere V, Singh K (1977) A glutamatergic corticostriatal pathway. Brain Res 128: 369–373
Meana JJ, Herrera-Marschitz M, Brodin E, Hökfelt T, Ungerstedt U (1991) Simultaneous determination of cholecystokinin, dopamine, glutamate and aspartate in cortex and striatum of the rat using *in vivo* microdialysis. Amino Acids 1: 365–373
Morino P, Herrera-Marschitz M, Meana JJ, Ungerstedt U, Hökfelt T (1992) Immunohistochemical evidence for a crossed cholecystokinin pathway in the rat. Neurosci Lett 148: 133–136
Morino P, Herrera-Marschitz M, Castel MN, Ungerstedt U, Varro A, Dockray C, Hökfelt T (1994) Cholecystokinin in cortico-striatal neurons in the rat: immunohistochemical studies at the light and electron microscopical level. Eur J Neurosci 6: 681–692
Mutt V, Jorpes JT (1968) Structure of porcine cholecystokinin-pancreozymin. 1. Cleavage with thrombin and with trypsin. Eur J Biochem 6: 156–162
Ouimet CC, Miller PE, Hemmings HC Jr, Walaas SI, Greengard P (1984) DARPP-32, a dopamine- and adenosine 3':5'-monophosphate-regulated phosphoprotein enriched in dopamine-innervated brain regions. III. Immunocytochemical localization. J Neurosci 4: 114–124
Peters A, Miller M, Kimerer LM (1983) Cholecystokinin-like immunoreactive neurons in rat cerebral cortex. III. Numerical considerations. Neuroscience 8: 431–448
Pettersson E, Herrera-Marschitz M, Rodriguez-Puertas R, Xu Z-Q, Hughes J, Elde RP, Ungerstedt U, Hökfelt T (1996) Evidence for aspartate-immunoreactive neurons in the neostriatum of the rat: modulation by the mesencephalic dopamine pathway via the D1 subtype of receptor. Neuroscience 74: 51–66
Reubi JC, Cuénod M (1979) Glutamate release *in vitro* from corticostriatal terminals. Brain Res 176: 185–188
Savasta M, Palacios JM, Mengod G (1988) Regional localization of the mRNA coding for the neuropeptide cholecystokinin in the rat brain studied by in situ hybridization. Neurosci Lett 93: 132–138
Snyder GL, Fisone G, Morino P, Gundersen V, Ottersen OP, Hökfelt T, Greengard P (1993) Regulation by the neuropeptide cholecystokinin (CCK-8S) of protein phosphorylation in the neostriatum. Proc Natl Acad Sci USA 90: 11277–11281
Vanderhaeghen JJ, Signeau P, Gepts W (1975) New peptide in the vertebrate CNS reacting with antigastrin antibodies. Nature 257: 604–605

Vanderhaeghen JJ, Lotstra F, Vierendeels G, Gilles C, Deschepper C, Verbanck P (1981) Cholecystokinins in the central nervous system and neurohypophysis. Peptides 2: 81–88

Wank SA, Harkins R, Jensen RT, Shapira H, deWeerth A, Slattery T (1992a) Purification, molecular cloning, and functional expression of the cholecystokinin receptor from rat pancreas. Proc Nat Acad Sci USA 89: 3125–3129

Wank SA, Pisegna JR, deWeerth A (1992b) Brain and gastrointestinal cholecystokinin receptor family: structure and functional expression. Proc Nat Acad Sci USA 89: 8991–8995

You ZB, Herrera-Marschitz M, Brodin E, Meana JJ, Morino P, Hökfelt T, Silveira R, Goiny M, Ungerstedt U (1994) On the origin of striatal cholecystokinin release: studies with *in vivo* microdialysis. J Neurochem 62: 76–85

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