The Effects of Cholecystokinin on the *In Vivo* Release of Newly Synthesized [³H]Dopamine from the Nucleus Accumbens of the Rat¹

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Abstract

It has been reported that in the medial nucleus accumbens (NAc) there are nerve terminals which contain either the neuropeptide cholecystokinin (CCK) or the catecholamine dopamine (DA), as well as terminals which contain both. In this study, we have examined the action of CCK-peptides on the basal and potassium-evoked release of [³H]DA within this structure. The *in vivo* release of [³H]DA, newly synthesized from [³H]tyrosine, was measured by using the push-pull cannula perfusion technique.

It appeared that a large percentage of the [³H]DA released under resting conditions was dependent upon nerve impulse activity as it was found that tetrodotoxin, absence of extracellular Ca²⁺, and the inhibition of DA synthesis by α -methyl-*p*-tyrosine all decreased [³H]DA release by more than 50%. In addition, the potassium-evoked release of [³H]DA was found to be almost completely dependent upon extracellular Ca²⁺.

When sulfated CCK-octapeptide was administered into the NAc, it was found to increase the basal levels of [³H]DA released at concentrations of 2×10^{-8} and 2×10^{-7} M. However, at 2×10^{-6} M there was no longer an effect by this peptide. The unsulfated form was found to have no effect at a concentration which was maximally effective for the sulfated form. In contrast to its effects on the basal release of [³H]DA, sulfated CCK-octapeptide was found to attenuate the potassium-evoked release of [³H]DA from the NAc in a concentration-dependent fashion from 2×10^{-9} to 2×10^{-6} M. The unsulfated form of the octapeptide had no effect on evoked release.

Our results suggest that CCK acts to modulate the release of DA within the NAc *in vivo* in a complex manner, as it appears that the action of CCK depends not only on the concentration tested but also on the excitation state of the tissue during the testing period.

In recent years, a number of peptides have been found in the brain which were originally isolated from the gut. One such peptide is cholecystokinin (CCK). Immunohistochemical and radioimmunological studies have demonstrated that CCK is found in most regions of the brain, predominantly as the sulfated carboxy terminal octa-

peptide (CCK-8S) with trace amounts of other forms present (CCK-33, unsulfated CCK-8 (CCK-8US), and CCK-4) (Dockray, 1976; Larsson and Rehfeld, 1979; Beinfeld, et al., 1981) as well. CCK-8S is a strong neurotransmitter candidate based on evidence that: (a) it is synthesized and stored by specific neurons in the brain (Emson et al., 1980; Goltermann et al., 1981); (b) it is released under physiological conditions (Pinget et al., 1979) and can be inactivated after release (Deschodt-Lanckman et al., 1981); (c) there are specific CCK receptors located in regions where the peptide is present (Hays, et al., 1980; Zarbin et al., 1983); and (d) it, but not CCK-8US, can alter the firing rates of neurons when applied iontophoretically (Dodd and Kelly, 1981; Skirboll et al., 1981; Morin et al., 1983; White and Wang, 1984).

Recently, Hokfelt et al. (1980) reported that CCK was co-localized with the catecholamine dopamine (DA) in a subset of neurons in the A10 region of the ventral tegmental area of the rat. In studies which combined fluorescent dye retrograde tracing techniques with immunohistochemistry, it was demonstrated that these CCK/DA neurons project primarily to the medial aspects of the nucleus accumbens (NAc) and olfactory tubercle (Hokfelt et al., 1980; Fallon et al., 1983). These findings were further supported by studies utilizing medial forebrain bundle transections and 6-hydroxydopamine lesioning of A10 DA cells in which CCK and DA were consistently depleted only in the medial NAc (Studler et al., 1981; Marley et al., 1982; Gilles et al., 1983). In addition, a recent report provides evidence that CCK might share the same storage sites as DA in some of the meso-accumbens terminals (Studler et al., 1984).

Since the first reports of CCK/DA coexistence appeared, there has been an increasing number of studies examining the interaction of CCK and DA in the brain. However, despite this increase in studies, the consequence of CCK-DA interaction within the brain has still not been elucidated. Behavioral studies have reported that CCK can increase, decrease, or have no effect upon DA-mediated behaviors such as stereotypy and locomotor activity (Diamond et al., 1983; Van Ree et al., 1983; Widerlov et al., 1983; Hamilton et al., 1984). Electrophysiological studies have demonstrated that CCK-8S is excitatory on some A9 and A10 DA cell bodies (Skirboll et al., 1981) as well as on medial NAc neurons (White and Wang, 1984) when applied by itself, yet it potentiates DA autoreceptor-induced inhibition of firing rate when administered immediately before the DA agonist apomorphine (Hommer and Skirboll, 1983). Results obtained in neurochemical studies are also contrasting, as one group has reported that CCK given into the NAc has no effect on DA turnover (Widerlov et al., 1983), whereas Fuxe et al. (1980) reported a decrease in DA turnover in the anterior portion of the NAc. In other studies, CCK has been reported to increase (Starr, 1982), decrease (Markstein and Hokfelt, 1984), or have little effect (Hamilton et al., 1984) on the release of [³H]DA from striatal slices *in vitro*.

As there seems to be a great deal of inconsistency within the literature regarding the interaction, if any, of CCK and DA, we have

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undertaken experiments designed to examine the interplay between these two compounds in an area where they coexist, the posterior NAc of the rat. In a previous paper (Voigt and Wang, 1984), we reported that CCK-8S attenuated the potassium-evoked release of endogenous DA from this region *in vivo*. In this study, we have examined the effects of CCK-8 peptides on the *in vivo* release of [3 H]DA newly synthesized from [3 H]tyrosine ([3 H]Tyr), under both basal and stimulated conditions. We chose to use newly synthesized [3 H]DA as a marker of dopaminergic activity within the NAc in this study for two reasons: (a) the enhanced sensitivity afforded by radioisotopic techniques makes it possible to measure DA release under resting conditions, and (b) that it appears to be newly synthesized DA which is preferentially released from nerve terminals (Besson et al., 1969).

Materials and Methods

CCK-8US and CCK-8S were obtained from Squibb and Sons, Inc. (Princeton, NJ), *d*-amphetamine was obtained from Smith, Kline and French (Philadelphia, PA), and L-[3,5- 3 H]tyrosine (specific activity, 53.8 Ci/mmol) was obtained from New England Nuclear (Boston, MA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals and preparations. Male Sprague-Dawley rats (SASCO Inc., St. Louis, MO) weighing 200 to 300 gm were maintained on a 12-hr light/dark schedule and had free access to food and water. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was incised, the skull was exposed, and a burr hole was drilled through the bone overlying the NAc. The dura was reflected back, and the cannula was lowered using the coordinates A 10.3, L 3.2, H -7.0, $\theta = 16^\circ$, with respect to lambda, based on the atlas of Paxinos and Watson (1982). An angular approach was used in order to avoid damage to the lateral ventricle. Anesthesia was kept deep by intraperitoneal injections of chloral hydrate as necessary, and body temperature was maintained between 36 and 38°C by using a heating pad (Fintronics Inc., Orange, CN).

Push-pull perfusion. Immunohistochemical studies show that, in the NAc, it is the caudomedial region which contains the highest density of CCK/DA terminals (Hokfelt et al., 1980). Thus, we positioned our cannulae within this region in order to observe the effects of CCK on DA in an area where coexistence occurs. The procedure used is the same as that described previously (Voigt and Wang, 1984) with the modification that [3 H]Tyr was present in the buffer (composed of (in millimolar concentration): NaCl, 126.5; NaHCO₃, 27.5; KCl, 2.4; KH₂PO₄, 0.5; CaCl₂, 0.75; MgCl₂, 0.83; Na₂SO₄, 0.5; glucose, 5.9; with the pH adjusted to 7.4 using 95% O₂/5% CO₂, v/v) at a concentration of 67 μ Ci/ml. This was accomplished by drying the needed amount of [3 H]Tyr in a vial under a stream of nitrogen, to which the necessary volume of buffer was added. In addition, the superfusates were collected as 10-min fractions in test tubes on ice containing 40 μ l of 0.5 M thioglycolic acid. At the end of the collection period, 1 ml of 95% ethanol was added to the tubes, which were then stored at -20°C until analysis, no later than 72 hr post-experiment.

[3 H]DA analysis. Superfusate fractions were adjusted to pH 6.5 to 6.9 using a 0.01 M Na-K phosphate buffer (pH 7.6) and were passed over an ion exchange column (Amberlite CG50), followed by alumina absorption chromatography using a previously published procedure (Westfall et al., 1976). 3 H-labeled catecholamines in the final eluate were quantified using liquid scintillation spectrophotometry. Using these techniques, the levels of 3 H-catecholamine recovered were 8 to 20 times the blank value (<0.15 nCi). As the NAc contains terminals from both DA- and norepinephrine (NE)-containing neurons (Veersleg et al., 1976), it was necessary to determine the composition of the newly synthesized 3 H-catecholamines recovered in the perfusate. Therefore, in some cases the final eluates from the alumina columns were evaporated to dryness using a Speedvac (Savant Instruments, Hicksville, NY) and were redissolved in 100 μ l of 0.4 N perchloric acid. These samples were then assayed for [3 H]DA and [3 H]NE content using a previously described high pressure liquid chromatography (HPLC) procedure (Voigt and Wang, 1984) with the additional step that 1-min fractions of the effluent stream were collected after having passed through the TL-5 electrode assembly. The radioactivity in these fractions was then quantitated using scintillation spectrophotometry and were plotted as a function of time, with the retention time of the radioactive peaks compared to those of known DA and NE standards. In brain superfusate fractions, >95% of the recovered 3 H-catecholamines coeluted with DA on HPLC (results not shown). Thus, the radioactivity recovered in alumina eluates is considered [3 H]DA.

Pharmacological manipulations. Drug treatments occurred 120 min after the start of the perfusion, which allowed for the release of [3 H]DA to reach a steady-state plateau. In experiments examining the effects of drugs on basal release, the indicated drug was given for a 10-min period in the superfusion buffer. Drug application was for 60 min in the cases of tetrodotoxin (TTX) and α -methyl-p-tyrosine (AMPT).

In experiments which examined the effects of drugs on evoked release, the following protocol was used. At 120 min after the beginning of perfusion, a 10-min pulse of high potassium buffer (56 mM K⁺, isotonically replacing Na⁺) was applied (S₁). This was followed by a 50-min period of perfusion with control buffer. At the end of this time, another 10-min pulse of high potassium buffer (S₂) with or without the test drug was administered. This stimulation period was followed by a second 50-min period of perfusion with control buffer. At the end of this period, the rat was removed from the stereotaxic device and histology was performed. In experiments testing the dependence of K⁺-evoked release on Ca²⁺, buffer without Ca²⁺ was perfused starting 10 min after S₁. For S₂, a high potassium buffer without Ca²⁺ was presented. This was followed by another 50 min perfusion with the Ca²⁺-free control buffer. All Ca²⁺-free buffers contained 1 mM EGTA.

Histology. After each experiment, the animal was removed from the stereotaxic apparatus and was perfused intracardially using phosphate-buffered formalin. The brains were then removed and kept overnight in formalin. Histological sections (50 μ m thick) were cut using a freezing microtome and mounted onto slides. Sections were then stained using cresyl violet and counterstained with neutral red.

Statistical methods. The manner in which data were calculated is based upon methods used by Nieoullon et al. (1977). The amount of [3 H]DA released during steady state differed from animal to animal, ranging from 1 nCi to 8 nCi. Thus, each animal served as its own control. The control (100%) value for each animal was calculated by taking the mean value of [3 H]DA released for the three fractions preceding the drug treatment. Release of [3 H]DA in each successive fraction was then expressed as a percentage of this control value. Data from animals were pooled and the mean $\pm$ SEM was calculated. Differences between control and drug-treated animals were then tested for statistical significance using a two-tailed Student's *t* test, where $p < 0.05$ was considered significant. In experiments where evoked release was measured using an S₂/S₁ ratio, the ratio was calculated for each animal with the results pooled according to experimental treatment. Once again, differences were analyzed using a two-tailed Student's *t* test, with $p < 0.05$ being taken as statistically significant. Animals in which the tip of the cannula was outside of the medial NAc were not included in the data analysis.

Results

Characterization of *in vivo* [3 H]DA release. As is shown in Figure 1, *in vivo* release of [3 H]DA from the NAc reached steady state within 90 min from the start of the experiment, and this steady state lasted throughout the course of the experiment, which in most cases was 200 min. As depicted in Figure 2, the level of basal [3 H]DA release appeared to be dependent upon the location of the cannula tip within the NAc. The highest degree of basal release was observed in the caudomedial NAc, followed by the anteromedial NAc. In experiments where the cannula was not in the NAc proper, recovered levels of [3 H]DA were at background levels or barely above background. Thus, the amounts of [3 H]DA recovered from various regions of the NAc correlated with the distribution of mesolimbic DA terminals within the NAc (Chronister, et al., 1980), suggesting that the push-pull system was recovering [3 H]DA that had been released from a discrete, localized area of the NAc.

Administration of AMPT, an inhibitor of the rate-limiting enzyme (tyrosine hydroxylase) of DA synthesis, resulted in a decrease in the release of newly synthesized [3 H]DA (Table I). Previous reports in the literature describe some mesolimbic neurons as being spontaneously active in anesthetized rats (Skirboll et al., 1981; Wang, 1981). Therefore, we sought to determine whether the basal release of [3 H]DA observed within the NAc was due to nerve impulse flow. Tetrodotoxin (TTX) is a compound which has been shown to block the propagation of action potentials along axons. When administered locally, TTX (at 1×10^{-5} M) was found to significantly decrease the basal release of [3 H]DA. Neurotransmitter release is thought to be a Ca²⁺-mediated process; therefore, we investigated the Ca²⁺ dependency of the basal release of [3 H]DA. Removal of Ca²⁺ ions from

Figure 1. Time course for release of newly synthesized $[^3\text{H}]\text{DA}$. $[^3\text{H}]\text{DA}$ was measured in 10-min fractions collected from the onset of the experiment. Values were calculated as described under "Materials and Methods." Steady-state plateau was reached by 90 min of perfusion. That the release observed was due to *de novo* synthesis of $[^3\text{H}]\text{Tyr}$ is demonstrated by the decrease observed when AMPT was added to the superfusion medium.

Figure 2. Distribution of $[^3\text{H}]\text{DA}$ basal release. This figure, obtained from a representative 26 animals, demonstrates that the degree of basal release of $[^3\text{H}]\text{DA}$ is dependent upon anatomical location. ●, <3.0 nCi/10-min fraction; ▲, 3 to 6 nCi/10-min fraction; +, >6 nCi/10-min fraction. CPu, caudoputamen; aca, anterior commissure. A 10.7 and A 10.2 refer to frontal sections from the atlas of Paxinos and Watson (1982).

TABLE I

Effects of various pharmacological manipulations on the release of newly synthesized [3 H]DA from the NAc

α -Methyl-D-tyrosine and tetrodotoxin were added to the perfusion buffer 120 min after the start of the perfusion, and were present for a minimum of 60 min. In the high potassium (K^+) experiments, two 10-min pulses of a buffer containing high K^+ (56 mM) were administered at 120 min (S_1) and 170 min (S_2) after the start of the perfusion. The S_2/S_1 ratio was then calculated by subtracting the mean amount of basal release from the three fractions preceding the S_1 or S_2 period from the amount of stimulated release present in either S_1 or S_2 , and then taking the ratio. For the Ca^{2+} -free experiments, Ca^{2+} -free buffer (either control or high K^+) containing 1 mM EGTA was perfused beginning at 130 min after the start of perfusion and continuing to the end of the experiment at $t = 200$ min. In the experiments on basal release, control values were 6.92 ± 2.0 nCi/10-min fraction ($n = 24$). In the high K^+ experiments, S_1 values were 38.95 ± 4.6 nCi/10-min fraction ($n = 21$).

Pharmacological Manipulation			Control (%)	N
α -Methyl-D-tyrosine	(100 μ M)		46 ± 10	3
Tetrodotoxin	(10 μ M)		67 ± 4	4
High Potassium	(56 mM)		563 ± 66 (S_2/S_1 : 0.93 ± 0.06)	21
Ca^{2+} -free buffer				
Basal			50 ± 11	4
Stimulated			13 ± 8 (S_2/S_1 : 0.11 ± 0.4)	4

the perfusion buffer resulted in a decrease in basal release (Table I), suggesting that a major portion of spontaneous release occurred via a calcium-dependent mechanism. This result, in combination with the effects of TTX and AMPT, suggests that roughly 50% of the basal release observed within the NAc occurred as a result of nerve impulse traffic.

Presentation of a buffer containing a high concentration of potassium (56 mM) for 10 min into the NAc resulted in a marked increase in the release of [3 H]DA (Table I). As a means of providing an internal control for each animal, an S_2/S_1 ratio protocol was used in examining the effects of compounds on the K^+ -evoked release of [3 H]DA. In control animals, an S_2/S_1 ratio of 0.93 ± 0.06 was obtained (see Fig. 4). As can be seen in Table I, omission of Ca^{2+} almost completely blocked the stimulatory effects of 56 mM K^+ during S_2 , demonstrating that this form of release was occurring via physiological processes.

Effects of CCK on release. Having found that the basal release of [3 H]DA was reflective of ongoing physiological processes, we proceeded to test the effects of CCK-8 peptides on this release by applying them locally. The effects of both the sulfated and unsulfated forms of CCK-8 were tested at various concentrations (Fig. 3). At the two lower concentrations, CCK-8S increased basal release in a dose-dependent fashion. At the higher concentration, release was not significantly different from that of control. Unsulfated CCK-8 had no effect on release in a concentration at which CCK-8S produced its maximal effect (2×10^{-7} M).

Experiments were also performed under conditions where [3 H]DA release was increased by introducing depolarizing levels of K^+ (56 mM). For these studies, CCK-8 peptides were added to the perfusion buffer immediately before their infusion during the S_2 period. CCK-8S caused a decrease in stimulated release at all concentrations tested. This inhibition exhibited a concentration dependency, with a greater inhibition being observed at the higher concentrations. Unsulfated CCK-8 had a minor effect which did not reach statistical significance at the concentration tested (Fig. 4).

Discussion

The results presented here extend our previous findings regarding the actions of CCK-8 peptides on the *in vivo* release of DA from the meso-accumbens terminals of the rat. Whereas in our earlier study we measured the release of DA from endogenous stores, in this study we have utilized the release of [3 H]Tyr as a marker of release. This protocol was used so that the release of DA under basal

Figure 3. Effects of CCK-8 peptides on the basal release of newly synthesized [3 H]DA. CCK-8 peptides were added to the superfusion buffer for 10 min at the indicated concentrations, as described under "Materials and Methods." At 20 nM, CCK-8S increased release to 155.8 ± 5.8 ($n = 5$) compared to control, whereas 200 nM CCK-8S increased release to 215.3 ± 26 ($n = 3$) compared to control. CCK-8S at 2 μ M and CCK-8US at 200 nM had no effect on basal release. **, $p < 0.01$, using two-tailed Student's t test. The numbers in parentheses are the number of animals tested.

conditions and the effects of CCK-8 peptides on this release could be quantified, which is something that was not possible in our earlier study. Additionally, this protocol also allowed us to examine more selectively the effects of CCK-8 peptides upon a particular compartment of releasable DA, that of the preferentially released pool of newly synthesized transmitter (Besson et al., 1969).

In our study, the [3 H]DA which was released was demonstrated to be done so by physiologically relevant processes, since local administration of AMPT or TTX, a compound that blocks the propagation of action potentials along axons, decreased the resting release of [3 H]DA, as did the lowering of extracellular Ca^{2+} . In addition to resting release, the release of transmitter evoked by high K^+ was also shown to be highly Ca^{2+} dependent (87%). The reduction by TTX of resting release might be due to a direct action upon dopaminergic axons, as previous studies have demonstrated that a portion of the mesencephalic DA cells which project to the NAc are spontaneously active (Skirboll et al., 1981; Wang, 1981). Combined, these results indicate that a large percentage of the [3 H]DA which is released within the NAc is not due to damage to terminals or to a nonspecific mechanism such as diffusion from intraterminal stores. Because the release we measured appeared to be of a physiological nature, we then examined the effects of CCK-8 peptides upon the release of [3 H]DA under both resting and stimulated conditions. Electrophysiological studies report that, in anesthetized rats, the percentage of NAc neurons which are spontaneously active is quite low (Pinnock et al., 1983; White and Wang, 1984). Thus, under conditions where there is a low degree of intrinsic neuronal activity within the medial NAc, CCK-8S was found to increase the amount of [3 H]DA release from meso-accumbens terminals. This was seen at the lowest concentration tested (20 nM), with the effect increasing

Figure 4. Effect of CCK-8 peptides on the K^+ -evoked release of newly synthesized $[^3H]DA$. As described under "Materials and Methods," CCK-8 peptides were added to the superfusion buffer during the S_2 period. Control values are 0.93 ± 0.06 . CCK-8S, when added to the buffer, attenuated release: S_2/S_1 values were: 2 nM, 0.71 ± 0.03 ; 20 nM, 0.61 ± 0.05 ; 200 nM, 0.66 ± 0.06 ; 2 μM , 0.52 ± 0.08 . In contrast, CCK-8US had no significant effect on release (0.78 ± 0.09). *, $p < 0.05$; **, $p < 0.01$ using a two-tailed Student's t test. Numbers in parentheses are the number of animals tested.

when a higher concentration (200 nM) was tested. Interestingly, at the highest dose tested (2 μM), there was no longer an effect by CCK-8S on $[^3H]DA$ release. That the action of CCK-8S was of a specific nature was demonstrated by the inability of CCK-8US to have any effect when tested at the maximally effective CCK-8S dose. This finding that the unsulfated CCK-8 lacked biological activity is in agreement with reports of other investigators (Skirboll et al., 1981; Morin et al., 1983; Markstein and Hokfelt, 1984), as well as with previous reports from our laboratories (Voigt and Wang, 1984; White and Wang, 1984). Proglumide, a putative CCK antagonist, was not tested because an earlier study in our laboratory (Wang et al., 1984) showed it to be ineffective in blocking the effect of CCK-8S on the evoked release of DA *in vivo*. In addition, a recent report has demonstrated that proglumide is not a competitive inhibitor of CCK-8S binding to brain binding sites (Knight et al., 1984).

A possible explanation for the biphasic effect of CCK-8S on the basal release of $[^3H]DA$ for the NAc can be formulated from recent electrophysiological studies examining the postsynaptic actions of these two compounds in this structure, in which it appears that CCK-8S and DA act as functional antagonists. Electrophysiological results demonstrate that CCK-8S causes excitation of a subpopulation of NAc neurons at low doses. At higher doses, the excitation becomes so strong that the cells enter into a depolarization blockade (i.e., inhibition of firing), as has also been reported for glutamate in the NAc (Pinnock et al., 1983; White and Wang, 1984). In contrast, it has been demonstrated that DA acts to inhibit NAc neurons (Woodruff et al., 1976; White and Wang, 1984). Thus, at low doses of CCK-8S, neuronal excitation might be countered by the increased release of an inhibitory transmitter, e.g., DA. As the dose of CCK-8S is increased, the degree of excitation would also increase, resulting in increased levels of DA release. At a high enough concentration of CCK-8S, neurons would become silenced through depolarization blockade, and the counterbalancing inhibition by DA would cease. Therefore, DA release would return to normal levels; this indeed is what we observed in our experiments examining the effects of CCK-8S on the basal release of $[^3H]DA$. Results from behavioral studies also demonstrate a biphasic effect of CCK-8S, in that low doses of this peptide (1 to 10 ng), when injected directly into the NAc, attenuate postsynaptic DA receptor-mediated hyper-

locomotor activity (Kelly et al., 1975; Van Ree et al., 1983), whereas at higher doses (3 to 10 μg) this peptide is no longer effective in modulating this behavior (Widerlov et al., 1983).

Next, we investigated the action of CCK-8 peptides on DA release evoked by a depolarizing stimulus (i.e., high K^+ buffer). Under these conditions, it appears again that CCK-8S acts to counterbalance DA. With respect to the K^+ -evoked release of $[^3H]DA$, CCK-8S was found to be inhibitory. This inhibition, which resembles that reported in an earlier study examining the release of endogenous DA from the NAc (Voigt and Wang, 1984), exhibited a more typical concentration-response curve, with greater inhibition observed with increased concentration. Once again, administration of CCK-8US did not result in any significant change in K^+ -evoked release. A similar action of CCK-8S on the stimulus-evoked release of $[^3H]DA$ from a brain region where the two coexist has also been reported by Markstein and Hokfelt (1984), who utilized cat striatal slices superfused *in vitro*. In contrast to our results are those of Hamilton et al. (1984), who reported that CCK-8S had no effect on the K^+ -evoked release of $[^{14}C]DA$ from rat NAc slices *in vitro*. However, these apparently discrepant results might be due to differences in the protocols utilized, i.e., *in vivo* superfusion versus a static *in vitro* method. As the effect of CCK-8S on the evoked release of $[^3H]DA$ observed in this study was similar to that reported for CCK-8S on endogenous DA in our earlier study, it does not appear that CCK-8S acts to regulate one transmitter pool preferentially.

The results from this study demonstrate that CCK exerts a complex set of actions upon DA-containing neurons. In addition, Meyer and Krause (1983) have shown that the opposite also holds true; i.e., DA has multiple effects upon CCK-containing neurons. Therefore, not only are the sulfation state of the peptide, the concentration being tested, and the state of excitation of the tissue being examined important, but the fact that the two substances act to influence one another's release and/or activity must also be taken into account. Further studies are needed in order to make a definitive statement concerning the result of CCK-DA interaction.

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