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A Caerulein-Sensitive Potentiation of the Behavioral Effects of Apomorphine by Dibutyryl-cAMP

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ELLINWOOD, E. H., JR., W. J. K. ROCKWELL AND N. WAGONER. *A caerulein-sensitive potentiation of the behavioral effects of apomorphine by dibutyryl-cAMP.* PHARMACOL BIOCHEM BEHAV **19**(6) 969–971, 1983.—The dopaminergic behavioral effects of apomorphine in rats were evaluated using a rating scale. Caerulein, a decapeptide physiologically similar to cholecystokinin, enhanced at lower doses and inhibited at higher doses the behaviors induced by apomorphine. Dibutyryl-cAMP, but not dibutyryl-cGMP, potentiated apomorphine behaviors. This potentiation was inhibited by a high dose of caerulein. These data provide evidence for an opposing effect of cAMP and caerulein or cholecystokinin in modulating dopaminergic systems.

Caerulein Cholecystokinin Apomorphine Dopamine Dibutyryl-cAMP Dibutyryl-cGMP Gastric peptides Rats

HOKFELT *et al.* [11] found cholecystokinin (CCK)-immunoreactive cells in three dopamine (DA) cell areas, i.e., the ventral tegmental area, the rostral substantia nigra compacta, and the pars lateralis. They also noted that many of the CCK cells contain tyrosine hydroxylase and that after 6-hydroxydopamine injection into the lateral hypothalamus, most tyrosine hydroxylase and CCK-immunoreactive fibers disappeared in the DA terminal areas: nucleus accumbens, tuberculum olfactorium, and bed nucleus of the stria terminalis. These findings are strong evidence for CCK being integrally linked with certain DA neurons and terminals.

Caerulein, a decapeptide [7], and cholecystokinin (CCK-8), a structurally and physiologically similar octapeptide, substantially inhibited high dose (50 mg/kg IP) methylphenidate-induced stereotyped gnawing in rats [20]. Caerulein was quite active, being approximately one-third to one-fifth as potent as haloperidol in blocking this DA-mediated behavior. Despite this robust caerulein and CCK-8 inhibitory effect on methylphenidate-induced behavior, the underlying pre- or postsynaptic mechanisms remain elusive. In recent presynaptic DA activity studies Fuxe *et al.* [8] reported that CCK fragments (1 nmol in 30 μl IVT in rats) reduced DA turnover in parts of the caudate and accumbens nuclei, whereas Kovacs *et al.* [14] reported an increased turnover (350 mg/kg of αMPT + 200 μg/kg CCK-8 IP in mice). Skirboll *et al.* [19] demonstrated that CCK (2–16 μg/kg IV in rats) increased DA cell firing in the substantia nigra and ventral tegmental areas (VTA), although a low concentration sometimes reduced firing in the VTA. Thus far, the reports on the relationship of CCK to central presynaptic dopamine effects lack an overall internal consistency, probably because of the variation in dose and route of administration, especially in light of the narrow window biphasic dose response relationship which is a major finding of this report.

Peripherally, caerulein and CCK stimulate amylase secretion by the pancreas and CCK stimulates contraction of smooth muscle [12,16]. Dibutyryl cyclic GMP (Bt_2cGMP) inhibits both of these actions [12], and inhibits CCK binding in rat pancreatic plasma membranes [13]. Furthermore, Bt_2cGMP stimulates and CCK inhibits feeding behavior in sheep [3]; in contrast, Bt_2cAMP stimulates pancreatic amylase secretion which is further augmented by CCK [15].

In fasted mice Saito *et al.* [17] found significant increases in CCK binding secondary to an increased number of CCK receptors in the olfactory bulb and hypothalamus, but not in other brain regions. Whole brain CCK levels are not changed with fasting [10,18].

If CCK receptors are altered in starvation, we questioned whether this may be related to the starvation facilitation of DA-mediated behaviors, locomotion and stereotypy [2]. To assess further the brain peptide-dopamine interactions involved, we have questioned: (1) In addition to the reported presynaptic interactions, is there a peptide effect on postsynaptic dopamine mechanisms, i.e., does CCK or the more potent caerulein inhibit apomorphine-induced behaviors? (2) Does Bt_2-cGMP, the peripheral CCK receptor antagonist, or Bt_2-cAMP alter apomorphine-induced activity? If so, is this alteration affected by caerulein?

METHOD

Male Sprague-Dawley rats weighing 230–260 g were im-

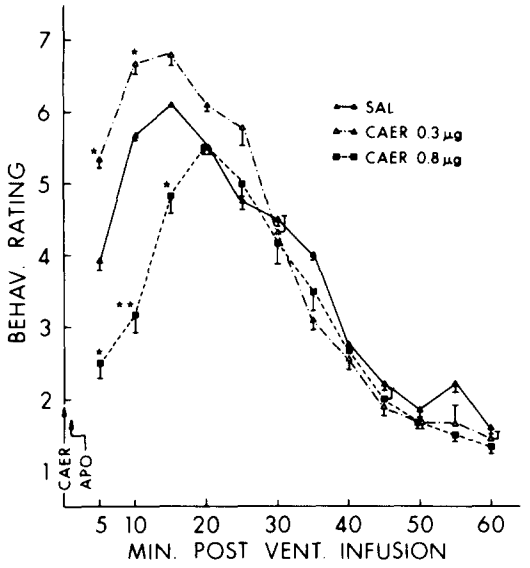

FIG. 1. Mean (±S.E.M.) effect of intraventricular saline (n=12), caerulein 0.3 μg (n=9) and 0.8 μg (n=6) on SC apomorphine (0.25 mg/kg)-induced behavior as a function of time following injections; differences from saline indicated by *$p<0.05$; **$p<0.01$.

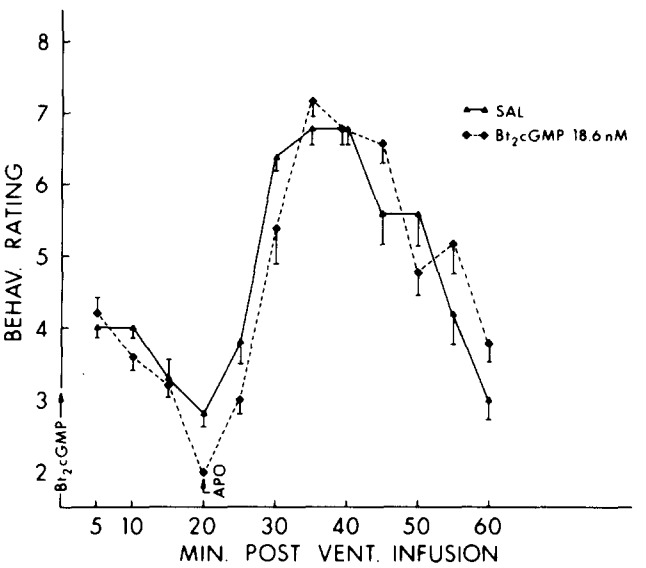

FIG. 2. Mean (±S.E.M.) effect of intraventricular Bt_2-cGMP 9.9 μg (n=7) and saline (n=5) on apomorphine (0.25 mg/kg)-induced behavior as a function of time following injections. There were no significant ($p>0.05$) differences at any time points.

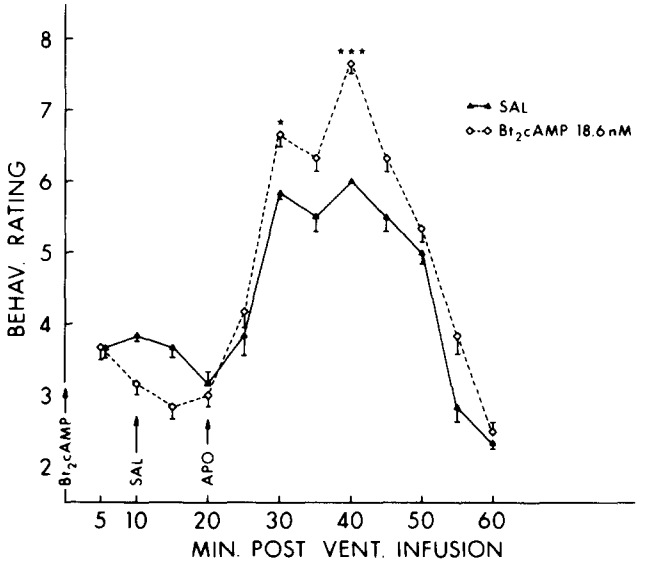

FIG. 3. Mean (±S.E.M.) effect of intraventricular Bt_2-cAMP 4.48 μg (n=6) and saline (n=6) on apomorphine (0.25 mg/kg)-induced behavior as a function of time following injections; differences between treatments indicated by *$p<0.05$; ***$p<0.005$.

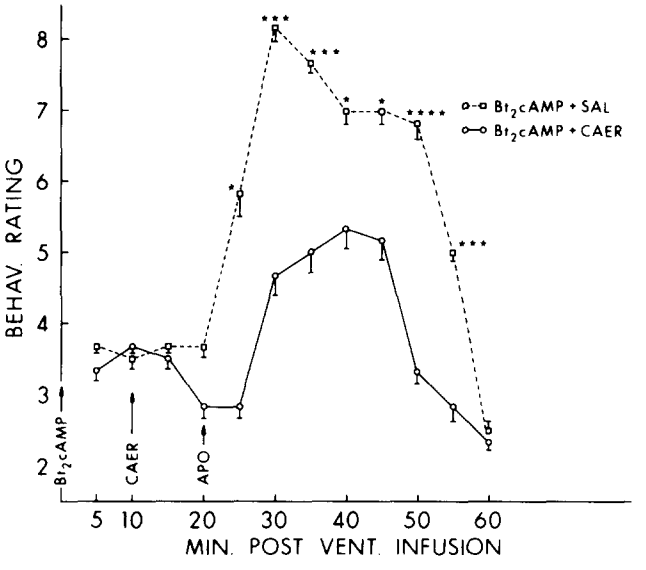

FIG. 4. Mean (±S.E.M.) effect of intraventricular caerulein 1.6 μg (n=6) and saline (n=6) on Bt_2-cAMP 4.45 μg facilitation of apomorphine (0.25 mg/kg)-induced behavior as a function of time following injections; differences between treatments indicated *$p<0.05$, ***$p<0.005$, ****$p<0.001$.

planted with a headpiece containing bilateral cannular guides (26 ga) extending into the lateral ventricle (see [4] for the method). The headpiece was configured and stereotaxically placed to allow the internal cannula to be positioned at A 5.4 mm, and VD 3.3 mm ventral to the dura (DeGroot Atlas).

Drugs administered were Bt_2-cAMP and Bt_2-cGMP sodium salt (Sigma Chemical Company) in saline infused in each ventricle in 0.2 μl increments every 30 sec for a total of 2.0 μl. Caerulein (Sigma) was instilled bilaterally in 0.2-μl increments every 12 sec for a total of 2.0 μl in each ventricle. Apomorphine hydrochloride (Merck) was injected subcutaneously as a 0.1% sodium metabisulfite aqueous solution. All drugs other than apomorphine were given intraventricularly to avoid variability in absorption kinetics. For each day the temporal order of drug administration is noted in the figures. In each figure the animals shown were divided into two groups (three for Fig. 1) and each group was administered the two treatments in alternate sequence. There was a 10-day interval between treatments.

Apomorphine (0.25 mg/kg)-induced behavior was as-

sessed using a rating scale specifically designed for dopamine-induced responses [6]. Student *t* tests were used to analyze differences between treatment effects.

RESULTS

In an initial experiment we found that intraventricular (IVT) caerulein at 0.3 μg potentiated apomorphine (0.25 mg/kg)-induced behavior, whereas a higher dose, 0.8 μg, inhibited the apomorphine effect (Fig. 1). Bt_2-cGMP (18.6 nmol IVT) had no effect on apomorphine-induced behavior (Fig. 2) and doses ranging from 5 to 75 nmol IVT failed to establish an effective dose (data not shown). In contrast, Bt_2-cAMP (18.6 nmol IVT) augmented apomorphine behavior at the time of peak apomorphine effect (Fig. 3). Caerulein (1.6 μg IVT) was effective in inhibiting the apomorphine Bt_2-cAMP-induced activity (Fig. 4).

DISCUSSION

The biphasic (facilitation/inhibition) caerulein dose effect on apomorphine behavior raises the possibility that gastric peptides have more than one mechanism of interaction with dopamine systems. Although the studies were designed to test postsynaptic interaction, the potentiation by the 0.3 μg IVT dose of caerulein on apomorphine may represent a facilitation of presynaptic dopamine release, since the small doses of apomorphine used induce submaximal behavioral effects and can still be augmented by presynaptic release. The overall effect could represent a caerulein neuromodulator inhibition or antagonist-like effect on both pre- and postsynaptic receptors, with the presynaptic sensitivity being greater. Kovacs *et al.* [14] reported that sulfated and nonsulfated CCK-8 (10^{-5} M CCK-8) potentiated potassium-induced release of [^{3}H] DA from striatal slices. Bt_2-cAMP stimulates tyrosine hydroxylase in striatal slices [1] and in soluble enzyme preparations [11], and reduces the inhibition of tyrosine hydroxylase by apomorphine [5]. In our studies neither caerulein (unpublished results) nor Bt_2-cAMP (see Fig. 3) increased motor activity prior to the administration of apomorphine, as might be expected with a potentiated dopamine release. Fuxe *et al.* [8] also did not find increased dopamine turnover following CCK IVT administration.

The substantial caerulein inhibition of apomorphine and Bt_2-cAMP potentiated apomorphine-induced stereotypy in the absence of an effect on predrug activity is difficult to interpret. Our studies do indicate that Bt_2-cGMP, the CCK antagonist in the periphery, does not have a postsynaptic dopamine modulating action on behavior. Overall, our data are consistent with the hypothesis that CCK serves a behaviorally significant modulating role on dopamine activity.

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