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PHORBOL ESTERS STIMULATE THE POTASSIUM-INDUCED RELEASE OF CHOLECYSTOKININ FROM SLICES OF CEREBRAL CORTEX, CAUDATO-PUTAMEN AND HIPPOCAMPUS INCUBATED IN VITRO

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SUMMARY: Incubation of slices of caudato-putamen, cerebral cortex and hippocampus for 5 to 15 minutes with phorbol 12,13-dibutyrate (PDB) or phorbol 12-myristate 13-acetate (PMA) increased potassium evoked cholecystokinin (CCK) release from 139% to 296% of control. The inactive 4 α phorbol and 4 α PDB did not alter CCK release. None of the active or inactive phorbols tested altered basal CCK release. These results suggest that there may be similarities in the regulation of CCK release in different brain regions. Although the physiological factors which regulate CCK release may differ in these tissues, it is possible that their common action is mediated by the products of inositol phospholipid turnover.

Cholecystokinin (CCK), a peptide common to brain and gut, has well-characterized hormonal activities in the gut (1) and may play a neurotransmitter or neuromodulator role in the brain (2). CCK concentration is very high in caudato-putamen (cp), cerebral cortex, hippocampus and other forebrain structures (3). The observation of co-localization of CCK and dopamine in some of the meso-limbic and nigral-striatal dopamine neurons (4), numerous studies of CCK's ability to modulate dopamine action (5) and its controversial clinical use in schizophrenia (6) has prompted studies on the regulation of CCK release from the terminal fields of these CCK neurons.

CCK release has been extensively studied. Purified synaptosome preparations are enriched in CCK and its release can be elicited from the same preparation or from rat brain slices with high potassium by a Ca^{++}-dependent mechanism (7). Numerous studies have examined the modulation of CCK release from cp. Dopamine D-2 agonists stimulated CCK release evoked by veratridine, while D-1 agonists inhibited (8-10). In posterior nucleus accumbens, D-2 agonists inhibit potassium-evoked CCK release (11). Vasoactive intestinal polypeptide (VIP) is a potent inhibitor of CCK release from cp (12).

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Other unknown substances, released by potassium along with CCK in the cp also inhibit CCK release (13). GABA A-receptor agonists (like muscimol) increased veratridine-induced release of CCK, while GABA A-receptor agonists (like baclofen) inhibited (14).

The experiments reported here on the effect of phorbol esters on the release of CCK were inspired by a previous observation that lithium treatment increases CCK release from the cp (15). Since lithium is known to block the turnover of inositol phospholipids (16), it was of interest to further examine whether the inhibitory action of agents which regulate CCK release from cp is mediated by the products of inositol phospholipid turnover. The products of inositol phospholipid turnover (in particular inositol triphosphate and diacylglycerol) are thought to be mediators of hormone or neurotransmitter action in many systems (17). Phorbol esters which mimic the action of diacylglycerol, are known to activate protein kinase C, resulting in the phosphorylation of specific proteins, with subsequent changes in cellular responses such as secretion (19-22). In addition to this action, in many systems they have a negative "feedback" action which uncouples or desensitizes the hormone receptor. Thus, in the presence of phorbols, calcium mobilization, inositol phospholipid turnover and stimulation of adenylate cyclase, which normally result from agonist or hormone receptor occupation, are inhibited (23-26).

MATERIALS AND METHODS

Release Experiments:

The tissues were removed from male Sprague-Dawley rats (200-250 g) obtained from Sasco (St. Louis). The details of the release experiments have been previously published (12,13). Some experiments used 40 mM potassium to induce release (Table I) while 60 mM potassium was used in others (Figures 1 and 2). The tissue to be tested was sectioned into 200 micron slices, and 4 or 5 slices were placed in each of four plastic baskets for the release experiments. Two baskets served as control, and two baskets received the drug to be tested. The phorbols were dissolved in DMSO and added to the slices at a final concentration of DMSO of 0.2%. Control slices received the same additions (without drug) of the same volume of DMSO. The slices were incubated with the drug or control solvent for 5, 10 or 15 minutes prior to a single basal and stimulation period of two minutes each. In this case, CCK release in basal period (if detected) was subtracted from CCK release in stimulus period, and the CCK release expressed as pg total CCK/vial in control slices was compared to drug-treated slices with the Student's t-test. The basal CCK release (when it was detectable) was from 10-25% of the release in the presence of potassium.

The volume of the release media for each basket was 0.75 ml and 0.2 ml was taken in duplicate to measure CCK release with the CCK RIA (3).

Materials:

The chemicals were all reagent grade. The phorbols were all purchased either from Sigma Chemical, St. Louis, MO or LC Services Corporation, Woburn, MA. The phorbols were dissolved at 1 mg/ml of DMSO, and aliquots were kept frozen in the dark and discarded after a single use.

RESULTS

Incubation of slices of cp, cerebral cortex, or hippocampus for 15 minutes with the active phorbol esters PMA and PDB prior to stimulation with potassium caused a highly significant increase in release of CCK evoked by 40 mM potassium (Table I). The

Table I

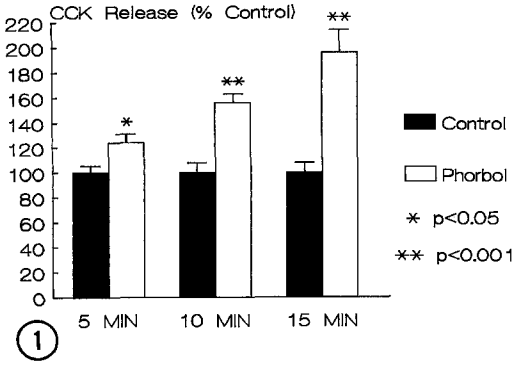
Effect of PMA, PDB, and 4 αphorbol on the release of CCK from caudatoputamen, cerebral cortex, and hippocampus

Brain Region	CCK Release (% control)					
	Drug Concentration					
	PMA 10^{-7}	PMA 10^{-6}	PDB 10^{-7}	PDB 10^{-6}	4 α10^{-7}	4 α10^{-6}
Caudatoputamen	296 ± 23.7*	149.5 ± 5.3**	239.7 ± 17.9**	138.6 ± 3.1**	102.3 ± 40.7	N.D.
Cortex	N.D.	215.4 ± 5.7***	N.D.	198.6 ± 12.5*	N.D.	66.4 ± 20.2
Hippocampus	N.D.	155.3 ± 6.4***	N.D.	240.3 ± 12.1*	N.D.	97.4 ± 8.3

N.D. = not determined, * = p < .02, ** = p < .01, *** = p < .001

magnitude of this increase was from 139% to 296% of control. The inactive phorbol 4 α did not alter the release of CCK (Table I). This stimulatory action of PMA and PDB requires some preincubation, as addition of the phorbol (at the same concentration as Table I) during the second two-minute basal and stimulation period did not alter the release of CCK (data not shown). The time course of PDB stimulation of CCK release from slices of cerebral cortex induced by 60 mM potassium is shown in Figure 1. Preincubation for 15 minutes produced the largest stimulation of CCK release, while shorter periods (5 and 10 minutes) produced a smaller but statistically significant increase in CCK release.

The effect of a wider range of doses of PDB on basal and potassium-induced CCK release was examined in cerebral cortical slices with a 15-minute preincubation (Figure 2). PDB from 10^{-9} to 10^{-6}M significantly elevated potassium-induced CCK release with the greatest effect observed at 10^{-7}M. Basal CCK release was not altered by any dose of PDB. The inactive 4 α isomer of PDB did not alter basal or potassium-induced release at any of these doses (data not shown).



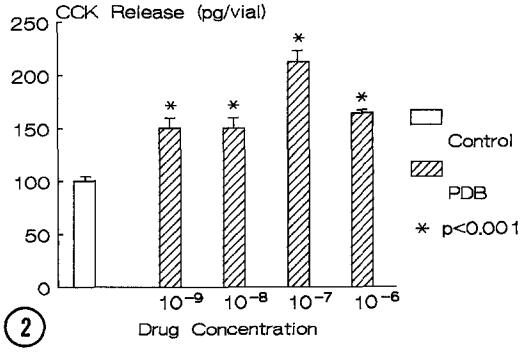


Fig. 1. Effect of the time of preincubation of slices of cerebral cortex with 10^{-7}M PDB on CCK release induced induced by 60 mM potassium.

Fig. 2. Effect of different concentrations of PDB on the 60 mM potassium-induced release of CCK from slices of cerebral cortex.

DISCUSSION

CCK release from cp, cerebral cortex and hippocampus elicited by potassium is significantly elevated by pretreatment with phorbol esters known to activate protein kinase C. The phorbols had no effect on basal CCK release, but were synergistic with potassium. Partial depolarization with potassium may be required to see the synergism because protein kinase C activation requires Ca^{++}, and potassium depolarization increases Ca^{++} influx. Interestingly, even at 60 mM potassium (the concentration of potassium which yields maximal depolarization-induced CCK release) phorbol esters are still capable of increasing CCK release up to 300%. The phorbols are not secretagogues, per se, but require time to work, as addition of the phorbol only during the basal and stimulus period does not significantly alter the release of CCK. This time requirement (about 5 minutes) may be related to the time it takes for the protein kinase C to travel to the plasma membrane prior to its activation (18). Phorbol esters have been shown to increase the release of peptide hormones in other systems: VIP (19), insulin (20) and somatostatin (21).

Elucidation of the mechanism of phorbol-stimulation of CCK release will require further experimentation. The most obvious explanation is that the phorbols are activating protein kinase C which is directly altering release. The mechanism of its alteration of release is unclear, but it may involve phosphorylation of a regulatory protein, alteration of an ion channel (27), or some other mechanism. However, based on our previous observation that lithium pretreatment (which is known to inhibit inositol phospholipid turnover) increases CCK release (15), it is tempting to speculate that the effect of phorbols on CCK release is related instead to the feedback inhibition of inositol phospholipid turnover that has been observed by phorbols in some systems (23,24,25).

That phorbols stimulate CCK release in three different brain regions suggest that the regulation of CCK release may have some similarities, despite the differences in the CCK cell types or innervation in these areas. CCK in the cp is present mainly in fibers and terminals, the cells of origin of this CCK is probably in the cerebral cortex, claustrum or amygdala (28). CCK in both the cerebral cortex and hippocampus is present mainly in interneurons (29), although it is likely that some of the CCK neurons in both areas project to other parts of the brain as well (30).

Although there may be similarities in the regulation of CCK release in the areas examined, the actual physiological agents which are responsible for this regulation in these tissues may not be the same. In previous studies, it was determined that CCK release from cp, but not from cerebral cortex was inhibited by VIP. Also, it was found that the unknown inhibitory substance released from both cerebral cortex and cp only inhibits CCK release from cp but not from cortex.

Further experiments (which are in progress) will be required to identify what the physiological regulator(s) of CCK release are in these brain areas and to identify the mediators of their action. Based on these results and the previous study, it is tempting to speculate that whatever these agents are, their inhibitory actions are mediated by the products of inositol phospholipid turnover.

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