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Brain Cortex Phosphatidylserine Inhibits Phosphatidylinositol Turnover in Rat Anterior Pituitary Glands (42146)

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Abstract. The *in vitro* effect of bovine brain cortex phosphatidylserine on ^{32}Pi incorporation into phosphatidylinositol, phosphatidylcholine, and phosphatidylethanolamine of rat anterior pituitary glands was studied. Phosphatidylserine (0.1 to 66.6 μM) decreased the incorporation of ^{32}Pi into phosphatidylinositol, but not phosphatidylcholine or phosphatidylethanolamine, in a concentration-related manner. The inhibitory effect of phosphatidylinositol was similar to that of dopamine in the same experimental conditions. The combined effects of submaximal concentrations of dopamine and phosphatidylserine elicited an apparently additive inhibitory effect on phosphatidylinositol synthesis. The inhibitory effect of phosphatidylserine was completely reversed by haloperidol and sulpiride and only partially by pimozide, antidopaminergic agents which per se do not affect phosphatidylinositol synthesis. The stimulatory effect of TRH to increase ^{32}Pi incorporation into phosphatidylinositol was decreased by phosphatidylserine. These observations suggest that the decrease in prolactin release in the presence of phosphatidylserine may be evoked through a dopaminergic mechanism.

Brain cortex phosphatidylserine (BC-PS), extracted and purified from bovine brain cortex, has been reported to exert several pharmacological actions in the central nervous system (1, 2). In particular, BC-PS stimulates the activity of tyrosine-hydroxylase in rat brain (3), induces dopamine (DA) release from rat striatum dopaminergic terminals (4), and stimulates DA-dependent adenylate cyclase in rat hypothalamus (5). Thus BC-PS can play a role in the activation of the adrenergic system in the hypothalamus, a brain area that BC-PS reaches in large amounts when systemically injected. Besides these actions at the CNS level, it has been observed that systemic administration of BC-PS reduces plasma prolactin level both in humans (6, 7) and in rats (8). This effect has been interpreted as being due to BC-PS interaction with the hypothalamic dopaminergic system, which in turn exhibits an inhibitory control on prolactin secretion (9, 10).

Recently, Canonico *et al.* reported that DA inhibits phosphatidylinositol (PI) turnover in rat anterior pituitary glands (11, 12). They proposed that the PI cycle could be an intracellular mechanism involved in the control of prolactin release in the rat and that changes in PI turnover could represent an early postreceptor event leading to the inhibition of prolactin secretion by DA. PI metabolism, in fact, is frequently associated with the activation of many membrane receptors in several tissues including the anterior pituitary gland [for reviews see (13–15)]. Changes in pituitary PI turnover may regulate the secretory processes initiated by certain hypothalamic hypophysiotropic hormones such as TRH (16, 17), GnRH (81), GRF (19), vasopressin (20), and, as already cited, DA (11, 12).

The present study was undertaken to investigate whether the effect of BC-PS on prolactin secretion could also be ascribed to modifications of PI turnover at the pituitary levels, as it was suggested for DA. The effect of BC-PS on the incorporation of ^{32}Pi into PI and other phospholipids of rat anterior pituitary glands has been studied. The experiments were performed also in the presence of DA and dopaminergic antagonists in an attempt to clarify the relationship between BC-PS and the hypothalamic dopaminergic system.

Materials and Methods. Adult female Sprague–Dawley rats (200–300 g body wt) were used since the proportional number of lactotrophs in pituitary glands is much greater

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in females than in males (21, 22). Animals were housed in a light and temperature controlled environment for approximately 1 week prior to the experiments. They were decapitated between 0830 and 0930 and their anterior pituitary glands were separated from the neurointermediate lobes, hemisected, and weighed.

Two hemipituitaries from different animals were preincubated in flasks for 45 min in 1 ml of Medium 199 containing Hanks' salts and 1.4 g/l sodium bicarbonate. The glands were then incubated for 30 min in Medium 199 with 50 μCi/ml $Na_2H[^{32}P]O_4$ and the test drugs. Hemipituitaries were homogenized in 1 ml of 0.25 *M* sucrose, 0.05 *M* Tris buffer (pH 7.5) in a Potter–Elvehjem homogenizer. Homogenization and extraction procedures were performed at 0–4°C. Phospholipids were extracted and separated by TLC according to Bligh and Dyer (23) as modified by Garbus *et al.* (24). Radioactivity was determined using a toluene PPO, POPOP scintillation cocktail. Results are expressed as counts per minute of [^{32}P]phospholipid per milligram of pituitary and statistics were performed by analysis of variance: (Anova 2 × 2) factorial analysis. Each group contained four flasks. Test drugs (BC-PS, from Fidia) were prepared as follows: liposomes were obtained by adding appropriate amounts of 50 m*M* TRIS–HCl, pH 7.4, to the phospholipid and by sonicating for 8 min with a Bromson sonifier. After sonication, peroxide content, phospholipid composition, and pH of the dispersion were not changed. Titanium residues released from the probe were discharged by centrifugation at 10,000*g* for 10 min. A commercial preparation of thyrotropin-releasing hormone (Rilatin) containing 500 μg TRH/ml was used; all other drugs were from the best commercial sources available. $Na_2H[^{32}P]O_4$ (specific radioactivity 50–500 mCi/mmole phosphorus) was from New England Nuclear; silica gel type 60 thin-layer chromatography plates were from Merck Laboratories.

Each study was repeated three times.

Results. The effects of DA and BC-PS on the incorporation of ^{32}Pi into PI of rat anterior pituitary glands are shown in Fig. 1. In agreement with previous reports (11), DA (at μM concentrations) inhibits phosphate incorporation into PI: Fig. 1 a. Similarly, incubation of anterior pituitary glands with various concentrations of BC-PS resulted in a concentration-related inhibition of ^{32}Pi into PI. Conversely, no significant change in the ^{32}Pi incorporation into phosphatidylcholine (PC) or phosphatidylethanolamine (PE) occurred at any of the DA and BC-PS concentrations tested. In separate experiments a significant reduction in ^{32}Pi incorporation into PI by BC-PS was observed after 15 min of incubation.

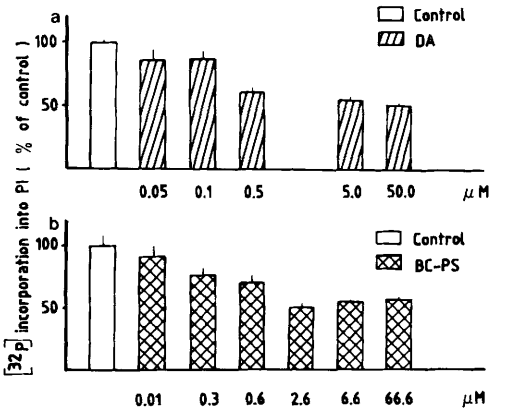

FIG. 1. *In vitro* effect of various DA and BC-PS concentrations on [^{32}P] incorporation into PI of female rat anterior pituitary glands.

Furthermore, when DA and BC-PS were tested together at submaximal concentrations (0.25 μM DA and 0.6 μM BC-PS) producing a limited inhibition of phosphate incorporation, their individual effects were additive (two-way analysis of variance; Fig. 2). The ^{32}Pi incorporation into PC and PE was not affected under these conditions. To characterize the action of BC-PS on PI turnover, we tested the effects of three DA receptor antagonists, haloperidol, pimozide, and sulpiride, at concentrations that block the inhibitory effect of DA on PI turnover *in vitro* (11). These concentrations were 50 n*M*, 50 n*M*, and 1 μM, respectively. The DA antagonists alone did not modify the phosphate incorporation into phospholipids of rat anterior pituitary glands (11), but blocked the effect of BC-PS (Fig. 3). Haloperidol and sulpiride produced a complete blockade while pimozide was partially active.

In contrast to the known prolactin release-inhibiting activity of dopamine, TRH is a

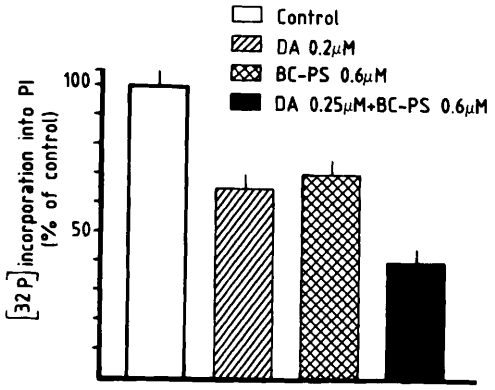

FIG. 2. *In vitro* effect of submaximal concentrations of DA and BC-PS on [^{32}P] incorporation into PI of female rat anterior pituitary glands.

prolactin-releasing factor. When rat anterior pituitary glands were incubated in the presence of various concentrations of TRH, a large increase of ^{32}Pi incorporation into PI occurred in concert with an increased release of prolactin in the medium (25). In our experimental conditions the effect of 0.1 μM TRH on PI turnover was significantly ($P < 0.05$) diminished in the presence of 0.6 μM BC-PS (Fig. 4). Again, phosphate incorporation into PC and PE was not modified.

Discussion. Modified PI turnover has been found in a variety of cell types, including endocrine cells, after stimulation of specific receptors by neurotransmitters and hormones (13, 26). In particular, PI turnover may control Ca^{2+} movements at the plasma membrane (13, 14) and it has been implicated in the regulation of those receptors acting predominantly through Ca^{2+} ions, such as those of the endocrine cells.

There is substantial evidence that DA directly inhibits prolactin secretion from the anterior pituitary gland via an interaction with specific receptors (27, 28). The precise mechanism by which the stimulation of these receptors leads to an inhibition of prolactin release remains to be established. Recently it has been found that DA was able to decrease specifically ^{32}Pi incorporation into PI in rat anterior pituitary glands (11, 12). This led to the suggestion that the PI cycle may constitute an intracellular mechanism controlling the hormone release in the rat and that changes in its cleavage and turnover may represent an early postreceptor event responsible for the regulation of prolactin release produced by factors such as DA. Numerous reports have characterized the pharmacological ac-

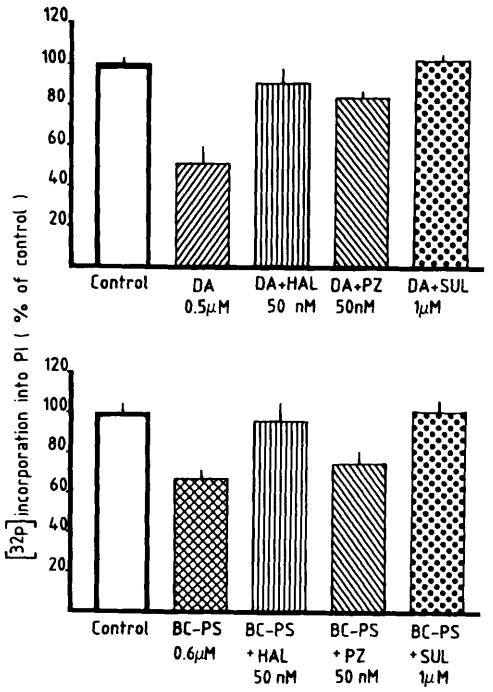

FIG. 3. *In vitro* effect of haloperidol (HAL), pimozide (PZ), and sulpiride (SUL) on the inhibition of [^{32}P] incorporation into PI by DA and BC-PS.

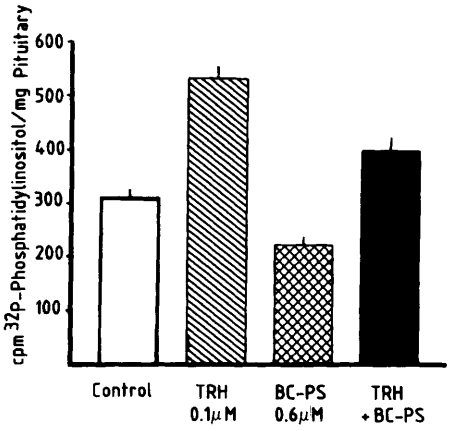

FIG. 4. *In vitro* effect of thyrotropin-releasing hormone (TRH) and BC-PS on [^{32}P] incorporation into PI of female rat anterior pituitary glands.

tions of BC-PS, from the activation of the catecholaminergic systems at the CNS level (1–5) to the production of hypoprolactinemia (8–10). The latter had been regarded as a consequence of the activation of the hypothalamic dopaminergic system exerted by BC-PS. This finding was also supported by the capacity of BC-PS to counteract the hyperprolactinemia induced by sulpiride and other neuroleptics. These findings provided the basis for the present study.

BC-PS inhibited ^{32}Pi incorporation into PI, in a concentration-related manner, apparently without affecting the metabolism of PC and PE. The effect was evident after 15 min incubation. This relatively slow response by hemipituitary glands is not surprising because of the time required to perifuse the entire system. This finding is in agreement with the data of Canonico *et al.* (11), who observed that the DA-induced decrease in phosphate incorporation into PI of rat anterior pituitary glands occurred only after 20 min of incubation, and with Young *et al.* (29), who demonstrated that the ACh-induced increase in ^{32}Pi incorporation in perifused bovine pituitary slices was apparent after 15 min.

The effect of DA on phosphate incorporation into PI by rat hemipituitaries is similar to the effect produced by BC-PS and together their effects are additive. These similarities are confirmed by the blockade that is produced by various dopaminergic antagonists on the action of BC-PS at the pituitary level. Haloperidol, sulpiride, and pimozide were able to counteract the effect of BC-PS, suggesting a direct DA mimetic action of the phospholipid. Identical effects are produced by these antagonists on prolactin release; although producing no change on prolactin release themselves at these concentrations, they are very effective to block the inhibitory effects of DA on prolactin release (6).

Another experiment showed that TRH abolishes the inhibition of phosphate incorporation induced by BC-PS, but, in turn, the TRH-dependent increase of ^{32}Pi incorporation is significantly reduced by BC-PS. Canonico *et al.* (25) have demonstrated the same phenomenon with DA, suggesting that the PI turnover might be a common mechanism through which TRH and DA control prolactin release at the pituitary level.

This new evidence, together with the previously characterized capacity of BC-PS to increase the DA-dependent adenylate cyclase activity and cAMP content in the hypothalamus, suggests a direct DA mimetic action of BC-PS. At present, however, it is not clear whether this is due to a direct interaction of BC-PS with the dopaminergic receptors or to some other interaction with the plasma membrane of the lactotrophs capable of influencing PI turnover.

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