INVOLVEMENT OF PHOSPHOLIPIDS IN THE COUPLING OF ADENOSINE AND DOPAMINE RECEPTORS TO RAT STRIATAL ADENYLATE CYCLASE

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Phospholipids are known to play an important role in the integrity of cell membrane and in the activity of membrane-bound enzymes. The involvement of membrane phospholipids in hormone-responsive adenylate cyclases in various tissues has been studied in many laboratories (Birnbaumer et al., 1971; Levey, 1971*a* and 1971*b*; Pohl et al., 1971; Rubalcava and Rodbell, 1973; Lad et al., 1979). The presence of adenosine and dopamine-sensitive cyclases in rat brain striatum has been reported earlier (Clement-Cormier et al., 1975; Premont et al., 1977; Anand-Srivastava and Johnson, 1980). It has also been reported that adenosine and dopamine stimulate adenylate cyclase by different mechanisms (Anand-Srivastava and Johnson, 1980). The present report explores phospholipid requirements for the regulation of adenylate cyclase by adenosine and dopamine and approaches the question of possible differences in modes of coupling of adenosine and dopamine receptors to the cyclase.

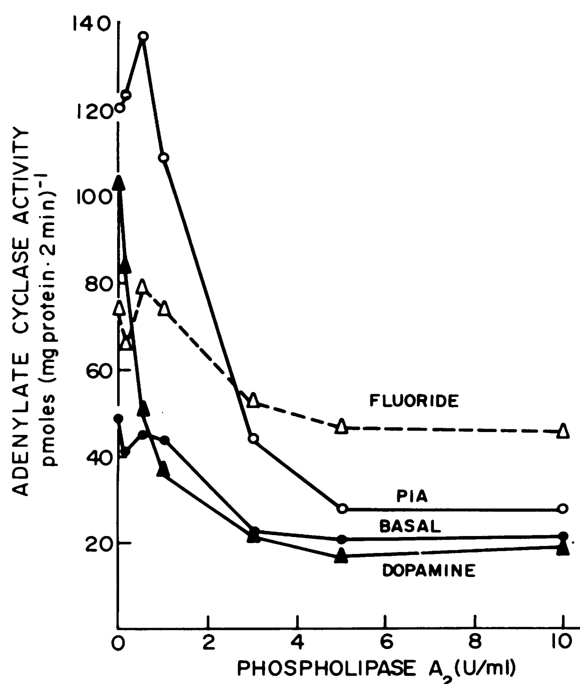

FIGURE 1 Effect of phospholipase A_2 treatment on adenylate cyclase activity of striatal washed particles. Activity was measured in the absence (-●-) and presence (-○-) of 100 μM PIA, 100 μM dopamine (-▲-), and 10 mM-NaF (-Δ-). The phospholipase A_2 treatment was performed at 20°C for 10 min. Adenylate cyclase activity was measured in the presence of 10 μM-GTP. Values are the averages of two experiments, each run in triplicate.

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RESULTS AND DISCUSSION

Striatal washed particles were prepared by the method described previously (Anand-Srivastava and Johnson, 1980). Adenylate cyclase activity was determined by measuring [^{32}P]cAMP formation from [α^{32}P] ATP by a modification (Walseth and Johnson, 1979) of the method described by Jakobs et al., (1976).

Fig. 1 shows the differential effects of phospholipase A_2

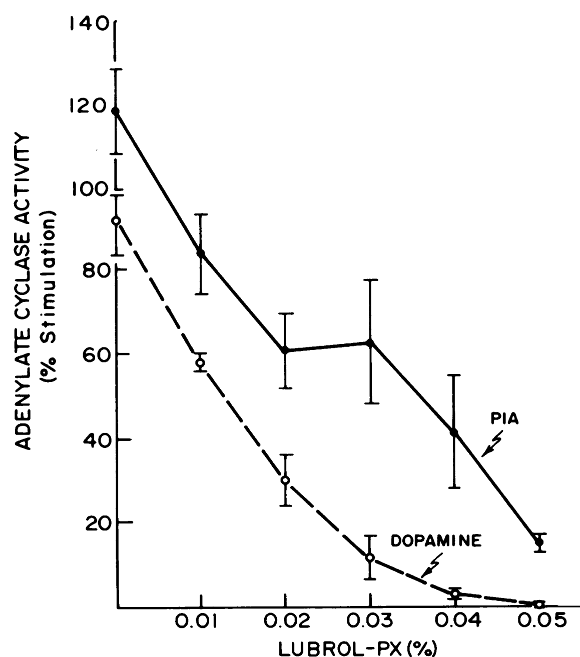

FIGURE 2 Effect of various concentrations of Lubrol-PX on striatal adenylate cyclase activity. Washed particles were incubated with various concentrations of Lubrol-PX at 20°C for 10 min. Particles were diluted to give a final concentration of 1.2 mg protein/ml to maintain constant protein:detergent ratios between experiments. Adenylate cyclase activity was measured in the presence of 100 μM PIA (-●-) or 100 μM dopamine (-○-). Values are means ± SEM of four different experiments, each run in triplicate.

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TABLE I
EFFECT OF ASOLECTIN ON ADENYLATE CYCLASE ACTIVITIES FROM LUBROL-PX-TREATED WASHED PARTICLES

Treatment	Adenylate cyclase activity		
	Basal	PIA 100 μM	Dopamine 100 μM
	pmol cAMP (5 min mg protein) − 1		
Control	163 ± 2	388 ± 5	314 ± 11
Control + asolectin (1%)	203 ± 1	463 ± 8	380 ± 10
16,000 *g* supernatant fraction	50 ± 1	78 ± 4	50 ± 4
16,000 *g* supernatant fraction + asolectin	83 ± 4	171 ± 3	73 ± 3
BioBead SM-2 fraction*	93 ± 13	207 ± 3	122 ± 4
BioBead SM-2 Fraction + asolectin (1%)	173 ± 5	465 ± 1	192 ± 22

Washed particles were treated with (0.03%) Lubrol-PX for 10 min at 20° and then were centrifuged at 16,000 *g* for 10 min. 16,000 *g* supernatant fraction was assayed for activity. All fractions were further incubated at 20° without and with 1% asolectin for 20 min and were used for adenylate cyclase assay. Values represent means ± SEM of triplicate determinations from one of two experiments.

*1 ml of the 16,000 *g* supernatant fraction was passed over BioBead SM 2 column (2 × 0.7 cm equilibrated and run with 10 mM imidazole, 1 mM EDTA buffer, pH 7.5) and assayed for cyclase activity.

treatment on adenosine and dopamine-sensitive cyclases. Phospholipase A_2 at a concentration of 1 U/ml completely abolished dopamine-sensitive cyclase activity without affecting N^6-phenylisopropyladenosine (PIA) sensitivity. The inactivation of dopamine-sensitive activity was reversed by subsequent treatment with asolectin or phosphalidylcholine, phosphatidylserine, or phosphatidylethanolamine (data not shown). Thus the data suggest that phospholipid plays an important role in the coupling process for dopamine activation of the cyclase, and that either the adenosine receptor or its coupling process does not require phospholipid or that the phospholipids involved in the coupling process are not affected by the treatments we used.

The conclusion from studies with the phospholipases are supported further by observations made with Lubrol-PX. Lubrol-PX completely abolished dopamine sensitivity under conditions causing only slight inhibition of sensitivity to PIA (Fig. 2). Moreover, Lubrol-PX extracted a PIA-sensitive but not dopamine-sensitive cyclase into a 16,000 *g* supernatant fraction (Table I). This was more evident once most of the free detergent was removed from this fraction by treatment with SM-2 Biobeads and by the addition of asolectin. The asolectin reversed any effect that Lubrol-PX may have had on PIA-sensitive adenylate cyclase but did not restore dopamine sensitivity. The dopamine receptor or the coupling process may have been irreversibly destroyed or separated from the cyclase under these conditions, whereas the coupling of the adenosine receptor remained largely intact.

Thus, adenosine receptors may be viewed as being more tightly coupled to the cyclase than are dopamine receptors and are thereby much less susceptible to modifications of the lipid environment of the membrane by phospholipases or detergents. Alternatively, the coupling process for dopamine and adenosine receptors and adenylate cyclase may occur by different mechanisms and involve different membrane constituents.

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