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PROTEIN KINASE C AND DOPAMINE RELEASE—II

EFFECT OF DOPAMINE ACTING DRUGS *IN VIVO*

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Abstract—The hypothesis that protein kinase C (PKC) plays a role in the release of dopamine (DA) in the nigrostriatal pathway was examined. It was found that injections of apomorphine, SKF 38393 (D1 agonist), LY 171555 (D2 agonist) or γ-butyrolactone (GBL) (which decreases impulse-induced release of DA) resulted in a decrease in particulate, and an increase in soluble, PKC activity. Injections of fluphenazine, haloperidol, SCH 23390 (D1 antagonist), sulpiride (D2 antagonist) or picrotoxin (γ-aminobutyric acid antagonist which increases DA release transneuronally) had the opposite effect of increasing particulate and decreasing soluble PKC activity. The total activity was not changed. These effects were receptor mediated since the effect of each agonist could be reversed by its specific antagonist. These drugs influenced PKC in the striatum in a dose-dependent manner. In contrast, no effects were seen in the cerebellum, a region with sparse dopaminergic innervations. The change in PKC activity was mediated via a change in the K_m for calcium, while the V_{max} was unchanged. The phosphorylation of endogenous substrate proteins by PKC was also altered by injections of these drugs. Besides affecting PKC, these DA acting drugs also affected the calmodulin-dependent protein kinase activity, but the direction of change was opposite to that for PKC. In a synaptosomal preparation, PKC acting drugs also affected the depolarization-induced release of DA. Adriamycin and melittin decreased the potassium-induced release of DA, whereas tetradecanoyl-phorbol-13-acetate (TPA) enhanced this release. These results showed that there was a good correlation between the ability of drugs to alter the impulse-induced release of DA *in vivo* and their ability to affect changes in particulate and soluble PKC activity. They lend support to the hypothesis that PKC, together with calmodulin, plays a key role in the release of DA in the nigrostriatal pathway.

Protein kinase C (PKC) has been implicated in the transduction of extracellular signals involved in the release of neurotransmitters [1]. This hypothesis is supported by the findings that phorbol esters, which activate PKC activity, can enhance the release of dopamine (DA), norepinephrine and acetylcholine from neuronal cultures and striatal slices *in vitro* [2–4]. In the present study, this hypothesis is examined by monitoring changes in PKC activity following injection of drugs which affect DA release *in vivo*. PKC activity is measured by the thiophosphorylation technique described in the preceding paper [5].

There are at least two subtypes of DA receptors; D1 and D2, which differ in their opposite linkage to adenyl cyclase [6]. They can be characterized pharmacologically by several selective drugs: LY 171555 (D2 agonist) [7] SKF 38393 (D1 agonist) [8], SCH 23390 (D1 antagonist) [9], and sulpiride (D2 antagonist) [10]. Apomorphine and haloperidol act on both D1 and D2 sites. The effects of these "selective" and "non-selective" dopamine acting drugs on the compartmentation of PKC activity in the striatum were determined in this study. The specificity of these drug effects was evaluated in terms of their dose-dependency, reversibility and regional localization. The mechanism by which PKC was altered was examined by analyzing the kinetic parameters for calcium, and the endogenous substrate proteins that were affected by these drug treatments. Furthermore, the involvement of the calmodulin (CaM)-dependent protein kinase was assessed and compared to the changes in PKC activity. These results together lend support to the hypothesis that PKC plays a key role in the release of DA in the nigrostriatal pathway.

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METHODS

Male, Sprague–Dawley rats (200–300 g) were injected (i.p.) with apomorphine (0.5 mg/kg), LY 171555 (0.3–3 mg/kg). SKF 38393 (10–100 mg/kg), sulpiride (30–200 mg/kg), SCH 23390 (0.5–10 mg/kg), haloperidol (2.5 mg/kg), fluphenazine (10 mg/kg), γ-butyrolactone (GBL) (750 mg/kg) or picrotoxin (2.5 mg/kg) for 30 min and then killed. Various brain regions were dissected, homogenized and separated into soluble and particulate fractions which were then analyzed for PKC or CaM-dependent protein kinase activity as described in the preceding paper [5]. Results, expressed as picomoles thiophosphorylated per minute per milligram of tissue, were analyzed by Student's *t*-test (two-tailed).

Endogenous substrate proteins were thiophosphorylated and analyzed by sodium dodecyl sulfate

Table 1. Effects of drug injections on PKC activity in the striatum

Treatment	PKC activity			
	Soluble*	% Control	Particulate*	% Control
Control	5.22 ± 0.53 (21)	100	3.48 ± 0.30 (21)	100
Apomorphine	7.10 ± 0.26† (15)	136	1.60 ± 0.21† (15)	46
GBL	6.89 ± 0.44‡ (8)	132	2.05 ± 0.38† (8)	59
Fluphenazine	2.82 ± 0.42† (8)	54	5.05 ± 0.27† (8)	145
Haloperidol	3.86 ± 0.26‡ (15)	74	5.12 ± 0.35† (15)	147
Picrotoxin	3.55 ± 0.19† (8)	68	5.29 ± 0.33† (8)	152

Rats were injected (i.p.) with the drugs and then killed. Brain tissues were fractionated into soluble and particulate fractions and then analyzed for PKC activity as described in the text.
Values are means ± SEM; the number of experiments is given in parentheses.
* Expressed as picomoles phosphorylated per minute per milligram of tissue.
† $P < 0.01$ compared to control.
‡ $P < 0.05$ compared to control.

(SDS)-polyacrylamide gel electrophoresis followed by autoradiography as described in the previous paper [5].

To measure the effect of drugs on the potassium-induced release of DA *in vitro*, crude mitochondrial fractions (P2) were prepared from striatal tissues. The tissues were prelabeled with [^{3}H]DA (0.01 μM) by incubating at 37° for 10 min. At this low concentration, DA is taken up selectively into the DA terminals. The uptake was terminated by adding a 7-fold excess of buffer containing drugs of various concentrations. Two minutes later, potassium (30 mM) was added. Aliquots (150 μl) were then filtered to separate tissue from medium at 1-min intervals for 5 min. The radioactivities in the filter and filtrate were determined. The percent release was computed as radioactivity in the filtrate/sum of radioactivity in the filter and filtrate × 100%. The percent release due to potassium was calculated by subtracting the steady-state release in the absence of potassium from the total release in the presence of potassium.

RESULTS

Injections of direct or indirect dopamine acting drugs into rats induced a change in PKC activity in the striatum (Table 1). Treatment with apomorphine or GBL increased the soluble and decreased the particulate PKC activity, whereas haloperidol, fluphenazine or picrotoxin had the opposite effect of decreasing the soluble and increasing the particulate PKC activity. The basal activity was not changed.

Injection of the "specific" dopamine acting drugs had qualitatively the same results as the "non-specific" drugs (Table 2). Treatment with the D1 agonist (SKF 38393) or the D2 agonist (LY 171555) increased the soluble PKC activity and decreased the particulate PKC activity. In contrast, administration of the D1 antagonist (SCH 23390) or the D2 antagonist (sulpiride) decreased the soluble and increased the particulate PKC activity. The total activity (soluble plus particulate activity) was changed only slightly by these drug treatments (<25%). These effects on PKC activity were mediated through the dopamine receptor site since the effect of each agonist was reversed by its antagonist. For example, co-injection of sulpiride reversed the effect of LY 171555 on soluble and particulate PKC activity to control levels (Table 3). Likewise, injection of SCH 23390 reversed the effects of SKF 38393 to control activity (Table 3).

The change in PKC activity was mediated by a change in the apparent K_m for calcium; the V_{max} was

Table 2. Effect of specific DA acting drugs on PKC activity *in vivo*

Drugs	PKC activity				
	Soluble*	% Control	Particulate*	% Control	Total
None	5.22 ± 0.53 (21)	100	3.48 ± 0.30 (21)	100	8.70
LY 171555	9.92 ± 1.46† (8)	190	0.77 ± 0.38† (8)	22	10.69
SKF 38393	8.61 ± 1.23‡ (8)	165	1.53 ± 0.21† (8)	44	10.14
Sulpiride	3.86 ± 0.21‡ (8)	74	7.06 ± 0.73† (8)	203	10.92
SCH 23390	3.97 ± 0.26‡ (8)	76	6.09 ± 0.45† (8)	175	10.06

Rats were injected (i.p) with the various drugs. The striata were fractionated into soluble and particulate fractions and analyzed for protein kinase C activity as described in the text. Total activity is the sum of the soluble and particulate activities.
Values are means ± SEM; the number of experiments is given in parentheses.
* Expressed as picomoles phosphorylated per minute per milligram of tissue.
† $P < 0.01$ compared to the controls.
‡ $P < 0.05$ compared to the controls.

Table 3. Reversibility of the effects of DA agonists by their antagonists

Treatment	PKC activity			
	Soluble*	% Control	Particulate*	% Control
A. None	4.49 ± 0.11	100	5.58 ± 1.31	100
LY 171555	7.07 ± 0.89†	157	1.15 ± 0.51†	21
LY + sulpiride	4.37 ± 0.17	97	5.48 ± 1.59	100
B. None	5.51 ± 0.31	100	4.40 ± 0.63	100
SKF 38393	7.67 ± 0.33‡	139	1.29 ± 0.61†	29
SKF + SCH	5.45 ± 0.65	99	4.66 ± 1.27	106

Rats were injected with the various drugs, and their striata were analyzed for protein kinase C activity as described in the text. Results are means ± SEM (N = 3 experiments).

* Expressed as picomoles phosphorylated per minute per milligram of tissue.

† $P < 0.01$ compared to controls.

‡ $P < 0.05$ compared to controls.

Table 4. Effects of drugs on the kinetic parameters of soluble PKC

Treatment	K_m (μM)	% Control	V_{max} (pmol/min/mg tissue)	% Control	N
None	3.7 ± 0.5	100	11.2 ± 0.5	100	15
Apomorphine	1.7 ± 0.3*	46	11.3 ± 1.0	101	6
LY 171555	1.6 ± 0.2*	56	10.2 ± 0.6	91	13
SKF 38393	1.7 ± 0.4*	47	10.5 ± 0.6	94	5
Haloperidol	9.5 ± 2.4†	157	12.1 ± 0.6	108	4
Sulpiride	5.9 ± 0.4*	161	11.8 ± 0.9	105	4
SCH 23390	5.5 ± 0.7†	148	11.3 ± 0.6	101	4

Rats were treated with the various drugs and then killed. The striatum was fractionated into soluble and particulate fractions. The soluble fraction was analyzed for PKC activity in the presence of various concentrations of calcium (5–150 μM), phosphatidylserine (30 μg/ml) and diolein (3 μg/ml). Values for the apparent K_m the V_{max} are means ± SEM; N is the number of experiments.

* $P < 0.01$ compared to controls.

† $P < 0.05$ compared to controls.

Table 5. Effects of drugs on the kinetic parameters of particulate PKC

Treatment	K_m (μM)	% Control	V_{max}(pmol/min/mg tissue)	% Control	N
None	12.6 ± 2.1	100	10.4 ± 0.7	100	20
Apomorphine	41.2 ± 5.4*	326	15.2 ± 3.5	146	4
LY 171555	34.9 ± 1.4*	277	11.0 ± 0.6	106	11
SKF	29.0 ± 4.2*	229	11.9 ± 0.5	114	5
Haloperidol	6.3 ± 1.0†	50	11.1 ± 0.5	107	6
Sulpiride	3.8 ± 0.8*	30	8.9 ± 0.6	86	11
SCH 23390	5.6 ± 0.6*	44	9.7 ± 0.4	93	13

Protein kinase C activity was determined in the striatal particulate fractions of rats that had received injections of the various drugs. The conditions and analysis were the same as in Table 3 except that the concentrations of PS and diolein were 10 and 1 μg/ml respectively.

* $P < 0.01$ compared to controls.

† $P < 0.05$ compared to controls.

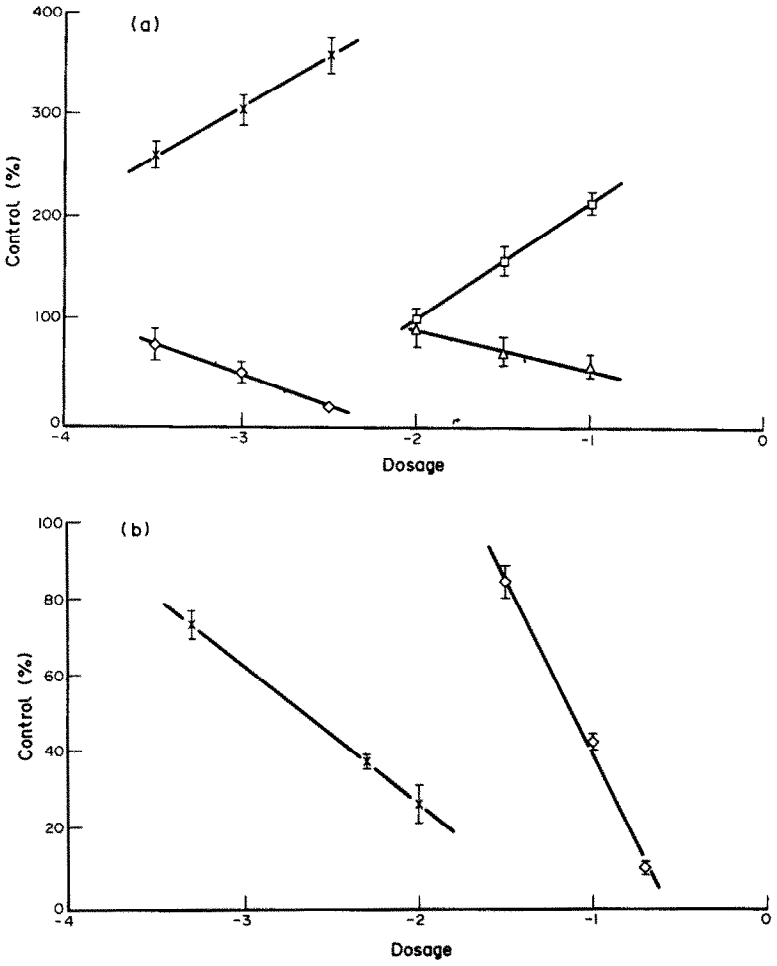

Fig. 1. Dose–response relationship of the effects on drugs on the apparent K_m of PKC for calcium. Rats were injected with various dosages of the drugs, and the striata wre then analyzed for soluble and particulate PKC activity, in the presence of lipids and various amounts of calcium in order to calculate the apparent K_m as described in the text. Results are expressed as percent of control K_m ± SEM vs log (dosage in g/kg) for (top panel) soluble (◇——◇) and particulate (×——×) fractions of the LY 171555 treated group, and the soluble (△——△) and particulate (□——□) fraction of the SKF 38393-treated group, (bottom panel) particulate fractions of the SCH 23390 (×——×) treated group, and the sulpiride (◇——◇) treated group.

unchanged (Tables 4 and 5). In the soluble fraction, LY 171555 and SKF 38393 significantly decreased the K_m by 44 and 53% respectively, while sulpiride and SCH 23390 increased the K_m by 61 and 49%, respectively (Table 4). In the particulate fraction, LY 171555 and SKF 38393 increased the K_m by 177 and 129%, respectively, while sulpiride and SCH 23390 decrased the K_m by 70 and 56%, respectively (Table 5).

The drug effects wre dose dependent (Fig. 1). The IC_{50} values for decreasing the K_m of soluble PKC by LY 171555 and SKF 38393 were approximately 0.8 and 100 mg/kg respectively. The K_m of the particulate PKC was doubled at about 0.1 mg/kg for LY 171555 and at 70 mg/kg for SKF 38393. The IC_{50} values for the decrease in the K_m for calcium for the particulate PKC following sulpiride or SCH 23390 injections were about 80 and 3 mg/kg respectively.

In contrast to these drug effects in the striatum, PKC activity was not affected in any way in the cerebellum. Here, there were no changes in the apparent K_m or the V_{max} for calcium for any of these drugs at various doses ranging from 0.3 to 100 mg/kg (Table 6).

The effects of these dopamine acting drugs on the calmodulin-dependent kinase were also examined. Injection of the DA agonists (apomorphine, LY 171555 and SKF 38393) led to an increase in calmodulin-dependent protein kinase activity, while injection of the DA antagonists (haloperidol, sulpiride and SCH 23390) resulted in a decrease in activity in the particulate fraction (Table 7). The direction of these changes was opposite to those seen with PKC.

The change in total phosphorylating activity was also reflected in the individual endogenous substrate proteins. In particular, injection of haloperidol increased the phosphorylation of the 17K and 21K

Table 6. Effects of drug injections on PKC activity in the cerebellum

Drug treatment	Soluble PKC		Particulate PKC	
	K_m (μM)	V_{min} (pmol/min/mg tissue)	K_m (μM)	V_{max} (pmol/min/mg tissue)
Control	1.8 ± 0.4	13.8 ± 0.8	1.3 ± 0.3	18.3 ± 0.5
LY, 0.3 mg/kg	1.7 ± 0.2	16.0 ± 0.8	1.5 ± 0.1	16.8 ± 0.5
LY, 1.0 mg/kg	1.8 ± 0.2	14.2 ± 0.2	1.3 ± 0.3	16.0 ± 1.0
LY, 3.0 mg/kg	1.6 ± 0.4	14.0 ± 0.1	1.4 ± 0.3	18.0 ± 2.0
SKF, 10 mg/kg	1.8 ± 0.4	13.3 ± 0.5	1.3 ± 0.2	20.0 ± 0.6
SKF, 30 mg/kg	1.8 ± 0.4	11.7 ± 0.7	1.3 ± 0.3	18.0 ± 0.6
SKF, 100 mg/kg	1.8 ± 0.3	12.8 ± 0.6	1.3 ± 0.2	20.0 ± 0.8
SCH, 0.5 mg/kg	2.3 ± 0.6	12.9 ± 0.6	1.4 ± 0.2	19.1 ± 0.8
Sulpiride, 100 mg/kg	1.7 ± 0.3	13.9 ± 0.1	1.3 ± 0.3	16.3 ± 0.5

Rats were injected with the various drugs and then killed. The cerebellum was then analyzed for PKC activity with various concentrations of calcium as described in the legend of Fig. 3.

proteins when tissues were incubated in the presence of calcium and lipid, while decreasing the phosphorylation of the 51K and 62K proteins in the presence of calcium and calmodulin (Fig. 2). The phosphorylation of the latter substrates was increased after apomorphine treatment.

The "specific" dopamine drugs had the same effects as apomorphine and haloperidol on endogenous phosphorylation. Injection of LY 171555 resulted in a decrease in the phosphorylation of the 17K and 21K proteins (Fig. 3). The effect was only seen at low concentrations of calcium (5 μM) and not at high calcium concentration. This is compatible with the observation above that these drugs affected the apparent K_m of the enzyme and not the V_{max}. Injection of SKF 38393 had the same effect as LY 171555. Injection of the DA antagonists, sulpiride or SCH 23390, increased the phosphorylation of the 17K and 21K proteins (Fig. 4).

The effects of PKC acting drugs on the potassium-induced release of DA from synaptosomes are shown in Fig. 5. The addition of tetradecanoyl-phorbol-13-acetate (TPA) increased the release, whereas adriamycin or mellitin decreased the release. These drugs had no effect on the steady-state release of DA.

Table 7. Effects of DA acting drugs on calmodulin-dependent protein kinase activity

Treatment	Activity	% Control
None	8.27 ± 0.70 (15)	100
Apomorphine	14.80 ± 0.74* (7)	179
LY 171555	11.99 ± 0.50* (4)	145
SKF 38393	11.16 ± 0.50* (4)	135
Haloperidol	5.71 ± 0.55† (5)	69
Sulpiride	5.95 ± 0.50† (4)	72
SCH 23390	6.45 ± 0.17† (4)	78

Rats were injected with the various drugs and then killed. The striatum was fractionated, and the membrane fraction was analyzed for calmodulin-dependent activity in the presence of calcium (5 μM) and calmodulin (50 units/ml) as described in the text.

Values are means ± SEM; (the number of experiments is given in parentheses.

* $P < 0.01$ compared to controls.

† $P < 0.05$ compared to controls.

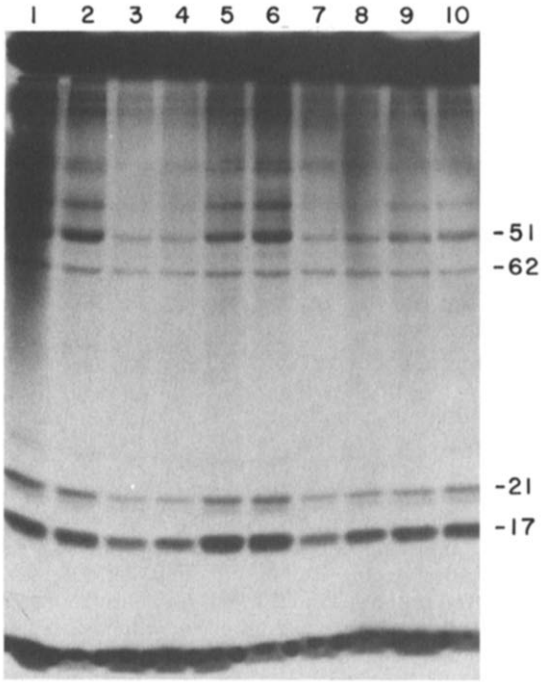

Fig. 2. Effect of apomorphine and haloperidol on the phosphorylation of endogenous substrate proteins by PKC and CaM-dependent protein kinase. Tissues from the control groups (lanes 1–3), apomorphine treated groups (lanes 4–7) and the haloperidol treated groups (lanes 8–10) were phosphorylated in the presence of calcium (15 μM) (lane 4), calcium plus 150 units of CaM (lanes 1, 5 and 8), calcium plus 500 units of CaM (lanes 2, 6 and 9) or calcium plus lipids (1 μg/ml diolein and 10 μg/ml PS) (lanes 3, 7 and 10) as described in the text. The numbers on the right indicate the $M_r \times 10^{-3}$. Note the difference in the phosphorylation of the 17K, 21K, 51K and 62K proteins.

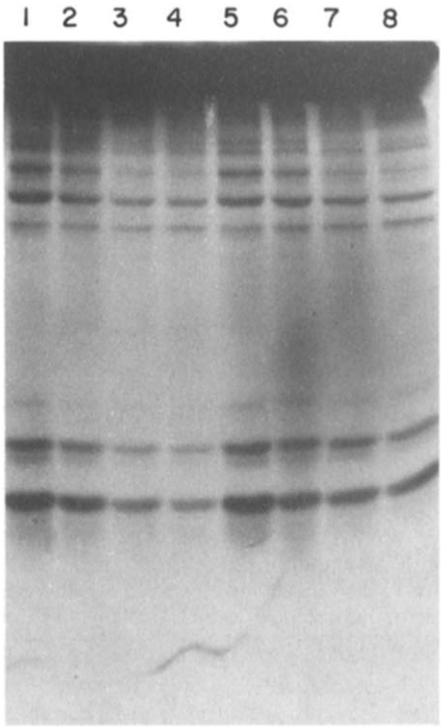

Fig. 3. Effect of LY 171555 treatment on endogenous phosphorylation by PKC. Tissues from control rats (lanes 5–8) and LY 171555 injected rats (lanes 1–4) were phosphorylated in the presence of lipids (1 μg/ml diolein, 10 μg/ml PS) and various amounts of calcium: 0, 5, 15 and 50 μM for lanes 4 and 8, 3 and 7, 2 and 6 and 1 and 5 respectively. Note the difference in the phosphorylation of the 17K and 21K proteins.

DISCUSSION

Utilizing the thiophosphorylation technique described in the preceding paper [5], this study found that injection of "non-selective" as well as "selective" dopamine acting drugs induced a differential change in soluble and particulate PKC activity in the striatum. These effects were dose dependent and receptor mediated. Each antagonist can fully reverse the effect of its agonist: SCH 23390 co-administration restored the effects of SKF 38393 to non-treated levels, and sulpiride attenuated the effects of LY 171555 to control activity. These effects were observed in the striatum, which is rich in DA receptors, and were not seen in the cerebellum where the dopaminergic innervation is sparse. These results suggest that stimulation of the DA receptor induced a specific change in PKC activity.

The significance of these findings is related to the hypothesis that PKC plays a key role in the release of DA *in vivo*. This is based on the observation that there was a good correlation between changes in PKC activity and changes in impulse-induced release of DA following drug injections. Systemic administration of the neuroleptics, which increase DA cell activity [11, 12] and DA release [13, 14] by blocking the DA receptor, or picrotoxin, which increases DA release indirectly by blocking the γ-aminobutyric acid (GABA) receptor [15, 16], resulted in an increase in particulate PKC activity. Injection of the DA agonists, which decrease DA cell activity [17] and DA release [18] by binding to the DA receptor, or GBL, which decreases DA release by decreasing the impulse flow in the nigrostriatal pathway [19], resulted in a decrease in particulate PKC activity. The effect of GBL is particularly interesting. Unlike the dopaminergic drugs, which act on DA receptors whose cellular location is not yet known, GBL acts specifically on the DA neuron by decreasing its firing rate [19]. The effects of GBL on PKC are, therefore, a direct consequence of the decrease in impulse-induced release of DA.

Other supporting evidence for the role of PKC in the release process rests on the finding that TPA, an activator of PKC, enhanced the depolarization-induced release of DA from synaptosomal fractions which is similar to that seen in neuronal culture cells [2–4]. Melittin and adriamycin, which are inhibitors of PKC, decreased the release of DA. However, these results should be interpreted with caution since melittin and adriamycin are not entirely specific for PKC but can also inhibit calmodulin-dependent kinase activity [20].

Another indication of the close relationship between PKC activity and DA release is the similarity in the potencies of the drugs influencing both processes. It has been reported that injections of apomorphine (0.1 mg/kg), LY 171555 (0.5 mg/kg) or SKF 38393 (100 mg/kg) decrease DA release by 20, 62 or 23%, respectively [14, 18], whereas injection of haloperidol (5 mg/kg), fluphenazine (1 mg/kg), sulpiride (50 mg/kg) or SCH 23390 (1 mg/kg) increase DA release by 130, 90, 30 or 30%, respectively [13, 14]. These doses are very similar to those eliciting the PKC response (see Fig. 1).

There is a large disparity in effective doses between LY 171555 and SKF 38393, and between sulpiride and SCH 23390 on PKC activity. This is probably due to a difference in pharmacokinetics, limiting penetration to the brain, rather than an intrinsic difference in receptor action since the order of potency of the agonists is not consistent with that of the antagonists. Furthermore, lisuiride (D2 agonist), apomorphine and SKF 38393 have been found to be equipotent when injected intracerebrally, even though there is a 5-fold difference in activity when injected systemically [21]. Despite this difference in availability, it is significant that these drugs affected DA release and PKC activity in the same dose range when they were administered via the same route, suggesting a close relationship between the two events.

Besides PKC, other protein kinases, such as the CaM-dependent protein kinase, have been implicated to play a role in the release process [22]. Injection of CaM-dependent protein kinase II into the presynaptic terminal of the squid stellate ganglion results in neurotransmitter release as measured by the electrophysiological responses [23]. In the CNS, the level of endogenous calmodulin is influenced by changes in dopaminergic activity. Induction of supersensitive DA receptors by chronic neuroleptic treatment results in an increase in calmodulin content in striatal membranes [24] and an increase in par-

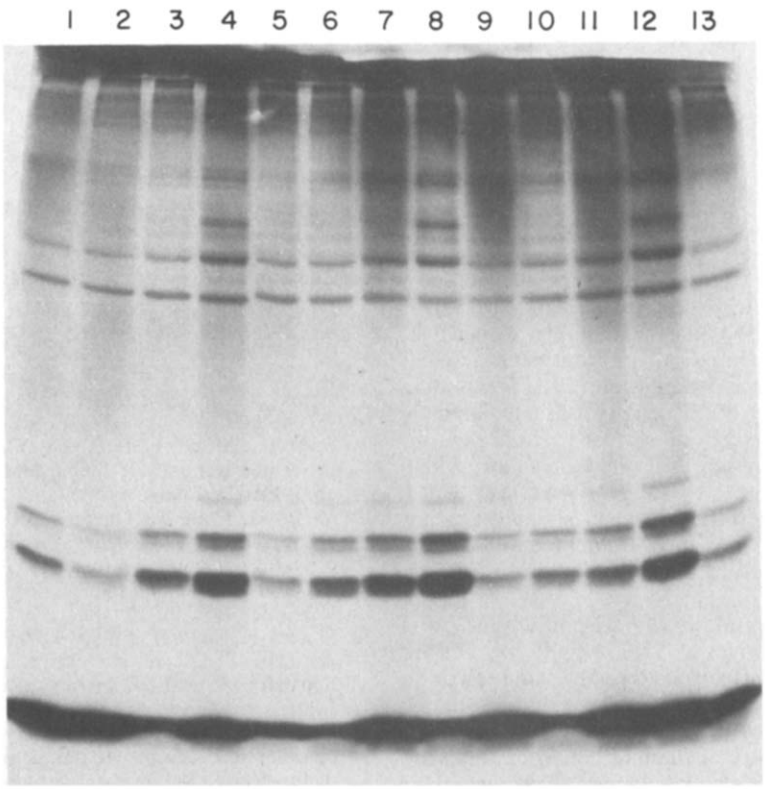

Fig. 4. Effects of SCH 23390 and sulpiride injections on endogenous protein phosphorylation by PKC. Tissues from the control group (lanes 1–4), the sulpiride treated group (lanes 5–8), and the SCH 23390 treated group (lanes 9–12) were phosphorylated in the presence of lipids (1 μg/ml of diolein, 10 μg/ml PS) and various amounts of calcium: 0 for lanes 1, 5, 9 and 13: 6 μM for lanes 2, 6 and 10; 15 μM for lanes 3, 7 and 11; 50 μM for lanes 5, 8 and 12. Note the difference in the phosphorylation of the 17K and 21K proteins.

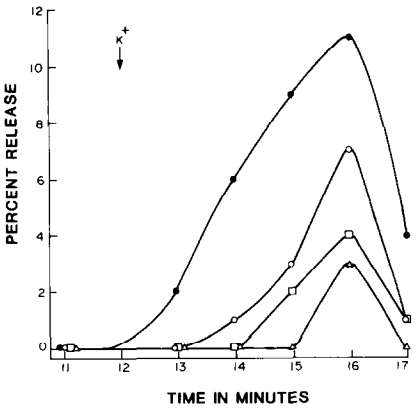

Fig. 5. Effects of drugs on the potassium-induced release of DA in synaptosomes. Striatal P_2 fractions were prelabeled with 0.01 μM [^{3}H]DA and then incubated with buffer (control, —O—), TPA (0.1 μM, ·——·), adriamycin (30 μM, □——□) or melittin (30 μM, △——△). Potassium (27 mM) was added at 2 min after drug addition as indicated by the arrow. At 1-min intervals, tissue aliquots were filtered and counted for radioactivity. Results are expressed as percent release induced by potassium over that of steady-state release. Each point is the mean of three determinations which varied less than 5%.

ticulate CaM-dependent protein kinase activity [25]. Conversely, desensitization of the DA receptor by prolonged exposure to dopamine *in vitro* results in a release of calmodulin from the membrane to the cytosol [26]. By inference, one would expect that normal tissues, exposed to DA via synaptic activity, should induce an increase in CaM-dependent kinase activity, and that treatment with the DA antagonists would have the opposite response of decreasing activity. This is exactly what was observed in this study. This finding provides evidence, for the first time, that acute DA acting drugs can affect CaM-dependent protein kinase activity, in spite of an absence of change in calmodulin levels [24]. The reason why calmodulin levels were not changed is not known, but it clearly did not prevent the change in CaM-dependent protein kinase activity following the injections of drugs. As pointed out by Nestler and Greengard [27], kinase activity is regulated physiologically by the concentration of intracellular free calcium and not calmodulin because the latter is present in excess in the cell. In this regard, it should be mentioned that these drug effects were evident only when a low calcium concentration was used in the analysis, since these drugs altered the apparent K_m of the enzyme without affecting the V_{max} (unpublished observation). The nature of the interaction between PKC and the calmodulin-depen-

dent protein kinase is not known, nor is it known if they operate at the same site. These results, however, do suggest that multiple mechanisms may be operative in the release process.

The molecular mechanism mediating the change in PKC activity following drug challenges is not known. Even though the drug treatments induced a differential change in soluble and particulate PKC activity, the total activity was unchanged. One mechanism that could account for this conservation of total activity is the translocation of the enzyme from the cytoplasm to the membrane pool and vice versa. Translocation of PKC has been shown to occur in tissue cultures incubated with phorbol esters [28] and following long-term potentiation [29]. The finding that the change in PKC activity was mediated by a change in the apparent K_m for calcium without affecting the V_{max}, however, makes it more likely that a change in enzyme configuration rather than a re-distribution of enzyme molecules would account for the effect. Precise measurement of PKC molecules by immunotitration will shed more light on this matter.

The cellular location of the D1 and D2 receptors mediating the changes in PKC activity has not yet been defined. They may occur on the same cell or on separate cells. They may be present on presynaptic autoreceptors or on postsynaptic intrinsic striatal neurons. The nature of the interaction between the D1 and D2 sites is also not known. However, their qualitatively similar effects on PKC activity contribute to the list of behaviors, such as the firing rate of basal ganglia neurons and stereotyped behavior in rats [30], where activation of D1 or D2 sites leads to a similar or synergistic response. Work is in progress to examine the nature of interaction between these DA sites.

In summary, the major finding of this study was that there is a correlation between the change in PKC activity by DA acting drugs and their ability to affect the impulse-induced release of DA *in vivo*. This correlation lends support to the hypothesis that PKC plays a key role in the release of DA in the nigrostriatal pathway.

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