Biochemical Pharmacology, Vol. 38, No. 24, pp. 4445–4454, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

PROTEIN KINASE C AND DOPAMINE RELEASE—III

EFFECT OF DOPAMINE DEPLETING DRUGS

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(*Received* 27 *December* 1988; *accepted* 19 *June* 1988)

Abstract—The hypothesis that protein kinase C (PKC) may modulate the release of dopamine (DA) in the nigrostriatal pathway was supported by findings that injections of drugs which affect DA release *in vivo* result in changes in PKC activity in the striatum (Giambalvo CT, *Biochem Pharmacol* **37**: 4009–4017, 1988). In the present study, it was found that the effects of the DA-acting drugs (apomorphine, LY 171555, SKF 38393, sulpiride, Sch 23390 and γ-butyrolactone) on PKC activity were prevented by prior diminution of the endogenous stores of DA with α-methyl-*p*-tyrosine (α-MT) or reserpine. This protective effect occurred at a dose and time when DA depletion was maximal, suggesting that the effects of the DA-acting drugs on PKC activity are dependent on the presence of an intact store of DA. Furthermore, since reserpine decreased the evoked release of DA, these results raise the possibility that PKC may be involved in the vesicular release process. Besides their effects on PKC activity, these depleting agents also prevented the DA-acting drugs from altering calmodulin-dependent protein kinase activity.

The hypothesis that protein kinase C (PKC†) plays an important role in the release of neurotransmitters [1], particularly of dopamine (DA) in the nigrostriatal pathway, has been examined in a previous study [2]. It was found that injections of drugs which stimulate the release of DA (haloperidol fluphenazine, Sch 23390, sulpiride or picrotoxin) result in a decrease in the soluble, and an increase in the particulate, PKC activity, while injections of drugs which inhibit DA release [apomorphine, SKF 38393, LY 171555 or α-butyrolactone (GBL)] have the opposite effect of increasing the soluble and decreasing the particulate PKC activity. These effects are dose-dependent, reversed by the specific antagonists, and specific to regions enriched in dopaminergic innervations. These effects are manifested as a change in the K_m for calcium without a change in the V_{max}, suggesting an allosteric alteration in enzyme structure. The phosphorylation of the specific endogenous substrates of PKC is also affected in a similar fashion by the drugs. These drugs also alter calmodulin-dependent protein kinase activity in the particulate fraction, but the direction of change is opposite to that of particulate PKC activity. These results raise the possibility that both PKC and calmodulin-dependent protein kinase may be involved in the modulation of DA release *in vivo*.

This possibility was assessed in the present study by examining the ability of drugs which deplete the endogenous stores of DA [α-methyl-*p*-tyrosine (α-MT) and reserpine] to prevent the changes in PKC activity induced by the DA-acting drugs. If PKC and calmodulin-dependent protein kinase are involved in the release of DA, then a decrease in DA release evoked by the pretreatments may abolish the effects of these drugs on the activities of the protein kinases. Furthermore, since reserping depletes only the vesicular pool whereas α-MT affects the cytoplasmic pool as well through synthesis inhibition, a comparison of their abilities to alter the kinase activities will yield information as to whether these kinases are involved in the cytoplasmic or vesicular release processes.

METHODS

Male, Sprague–Dawley rats (200–300 g) were injected (i.p.) with α-MT (0.1 to 0.8 g/kg) or reserpine (5 mg/kg) 20–180 min before being challenged with the DA-acting drugs: apomorphine (0.5 mg/kg), LY 171555 (1 mg/kg), SKF 38393 (30 mg/kg), sulpiride (100 mg/kg), Sch 23390 (0.5 mg/kg) or GBL (750 mg/kg). The rats were killed 30 min after these drug injections. The striata were dissected, homogenized, and separated into soluble and particulate fractions, each of which was analyzed for PKC activity in the presence of lipids and various concentrations of calcium to calculate the K_m and V_{max} as described previously [2, 3]. Briefly, each tissue fraction was incubated with calcium (1–50 μM), phosphatidylserine and diolein

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† Abbreviations: PKC, protein kinase C; DA, dopamine; Sch 23390, *R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol; LY 171555 (quinpirole), *trans*-(−)-4a*R*-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-1*H*(2*H*)-pyrazolo-(3,4-g)quinoline; SKF 38393, 1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride; GBL, γ-butyrolactone; and α-MT, α-methyl-*p*-tyrosine.

Table 1. Effect of α-MT on PKC activity

α-MT treatment (g/kg)	K_m (μm)	% Control	v_{max} (pmol/min/mg tissue)	% Control
I. Soluble PKC activity				
None	4.7 ± 1.5	100	9.5 ± 0.5	100
0.1	4.1 ± 1.8	87	8.5 ± 0.6	90
0.3	5.2 ± 1.9	112	7.6 ± 0.3*	80
0.6	4.5 ± 0.9	96	6.6 ± 0.5*	70
II. Particulate PKC activity				
None	12.8 ± 2.9	100	18.8 ± 1.5	100
0.1	15.4 ± 2.4	120	16.0 ± 1.0	85
0.3	12.6 ± 1.1	98	15.7 ± 1.7	83
0.6	11.4 ± 1.2	89	13.5 ± 0.1*	72

The indicated doses of (α-methyl-*p*-tyrosine (α-MT) were injected 3 hr prior to killing the rats. PKC activities in the soluble and particulate fractions were analyzed at various concentrations of calcium to calculate the K_m and V_{max} values, as described in the text. Results are expressed as mean ± SE (N = 4) for K_m and V_{max} and as percent of control activity.

* $P < 0.05$ vs control (Student's two-tailed *t*-test).

Table 2. Effects of α-MT pretreatment on changes in PKC activity induced by DA-acting drugs

	PKC activity (% of control)			
	Soluble		Particulate	
Treatment	K_m	V_{max}	K_m	V_{max}
(1) α-MT	94 ± 10	80 ± 4*	99 ± 10	75 ± 4*
(2) SKF	53 ± 5*	94 ± 6	276 ± 16†	102 ± 17
SKF + α-MT	98 ± 10	95 ± 6	105 ± 8	99 ± 19
(3) LY	48 ± 6*	96 ± 9	217 ± 13†	94 ± 12
LY + α-MT	90 ± 9	124 ± 17	95 ± 10	98 ± 15
(4) Sch	172 ± 11*	103 ± 2	35 ± 4†	96 ± 8
Sch + α-MT	109 ± 7	108 ± 7	97 ± 5	101 ± 6
(5) Sul	210 ± 10†	99 ± 6	23 ± 6†	100 ± 25
Sul + α-MT	110 ± 11	94 ± 7	104 ± 5	113 ± 13

Rats were pretreated with α-MT (0.6 g/kg) 3 hr prior to injection of the various DA-acting drugs. See Methods for doses of these drugs. Thirty minutes later, the rats were killed and the striata were analyzed for soluble and particulate PKC activities as described in the text. Abbreviations: α-MT, α-methyl-*p*-tyrosine; SKF, SKF 38393; LY, LY 171555; Sch, Sch 23390; and Sul, sulpiride. Results are expressed as percent of the control K_m and V_{max} (mean ± SE N = 4).

See Table 1 for control values.

* $P < 0.05$ vs control.

† $P < 0.01$ vs control.

Table 3. Dose-dependent effects of α-MT pretreatment on particulate PKC activity

	(I)		(II)		(III)	
Treatment	K_m (μM)	% Control	K_m (μM)	% Control	K_m (μM)	% Control
None	16.6 ± 1.6	100	12.9 ± 0.1	100	12.0 ± 2.2	100
Sch 23390	5.0 ± 0.1*	30	4.9 ± 1.5†	38	3.5 ± 0.9†	30
Sch + α-MT	8.6 ± 0.1†	52	11.7 ± 1.7	91	13.3 ± 0.6	112
α-MT	18.7 ± 0.8	113	13.5 ± 1.3	105	11.6 ± 0.4	97

Rats were pretreated with various doses of α-MT: (I) 0.4 g/kg for 3 hr, (II) 0.6 g/kg for 3 hr, and (III) 0.8 g/kg in two doses 3 hr apart. Sch 23390 (0.5 mg/kg) was then injected, and the rats were killed 30 min later. The striatum was dissected and the particulate fraction was analyzed for PKC activity at various concentrations of calcium to calculate the K_m as described in the text. Results are expressed as mean ± SE (N = 4) for K_m and as percent of control activity.

* $P < 0.01$ vs control.

† $P < 0.05$ vs control.

(Sigma Chemical Co., St Louis, MO) (30 and 3 μg/ml, respectively, for the soluble fraction; 10 and 1 μg/ml, respectively, for the particulate fraction), histones (17 μM, type III) and ATPγS (40 μM, 15 Ci/mol; New England Nuclear, Boston, MA) at 30° for 3 min. The reaction was stopped by adding 2 ml of 25% trichloroacetic acid (TCA). The precipitated proteins were filtered, rinsed three times with 1.25% TCA, and then counted for radioactivity. PKC activity was calculated as the net phosphorylating activity (expressed as picomoles thiophosphate transferred per minute per milligram of tissue) above basal activity, measured in the absence of added calcium or lipid. The kinetic parameters were calculated according to the method of Wilkinson [4]. The calmodulin-dependent protein kinase activity was measured as described previously [2, 3], using calcium concentrations of 1–50 μM and calmodulin (10 μg/ml; Sigma). The calmodulin-dependent kinase activity was calculated as the stimulation by calmodulin above basal activity which was measured with no added calcium or calmodulin. Results of the drug treatments were compared for significance by Student's two-tailed *t*-test. Phosphorylation of endogenous substrate proteins was analyzed by sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS–PAGE) and autoradiography as described previously [2, 3]. The autoradiograms were scanned with a densitometer linked to a Hewlett–Packard integrator, in order to calculate the peak area for each protein band as an index of the amount of radioactivity incorporated.

RESULTS

Injection of α-MT resulted in a small but dose-dependent decrease in the V_{max} of the soluble and particulate PKC activity in the striatum, without changing the K_m for calcium (Table 1).

Pretreatment with α-MT (0.6 g/kg 3 hr prior) attenuated the effects of SKF 38393 (D_1 agonist), LY 171555 (D_2 agonist), Sch 23390 (D_1 antagonist) and sulpiride (D_2 antagonist) on PKC activity (Table 2). Thus, the decrease in the K_m for calcium in the soluble fraction and its increase in the particulate fraction following treatments with the DA agonists were not seen in rats that had been pretreated with α-MT. Similarly, the increase in the K_m in the soluble fraction and its decrease in the particulate fraction following treatments with the DA antagonists were no longer apparent in the α-MT pretreated group. While none of these DA ligands altered the V_{max} for calcium, the decrease in the V_{max} seen with α-MT injection alone was also attenuated by the combined drug treatments.

The preventive effects of α-MT co-varied with the amount of DA depletion since a lower dose of α-MT (0.4 g/kg) resulted in a partial reversal of the effects of Sch 23390 whereas the higher doses (0.6 to 0.8 g/kg) totally reversed the effects (Table 3).

Injection of reserpine produced a time-dependent change in PKC activity. At 30 min, the K_m was increased in the soluble fraction and decreased in the particulate fraction (Table 4). The V_{max} was unchanged (data not shown). These effects were similar to these seen with injection of the DA antagonists. At 3 hr, when DA depletion is presumably maximal [5], there was no change in the K_m but the V_{max} was decreased in both the soluble and particulate fractions (Table 5), similar to results seen with α-MT.

The effects on histone phosphorylation were also reflected in the phosphorylation of endogenous substrates of PKC. As seen in Fig. 1, the phosphorylation of proteins by particulate PKC was increased at 20 min and decreased at 60 and 180 min following reserpine, when analyzed in the presence of 15 μM added calcium. No effect was seen at the lower calcium concentration (1 μM). Thus, the phosphorylation of the 17K protein, as measured by densitometry, was 163, 70 and 83% of control activity at 20, 60 and 180 min, respectively, after reserpine treatment. Similarly, the phosphorylation of the 21K protein was 154, 70 and 95% of control activity at 20, 60 and 180 min, respectively, after reserpine.

The effects of reserpine on PKC activity at 20 min may be due to an immediate decrease in DA levels at the autoreceptor site since they were abolished by co-administration of LY 171555 and potentiated by sulpiride (Table 4).

In contrast, 3 hr after reserpine treatment, the effects of both DA agonists and antagonists, as well as the indirect acting drug GBL, on PKC activity were attenuated (Table 5). These effects were similar to those seen with α-MT and presumably coincided with the maximal decrease in DA levels.

Reserpine pretreatment also attenuated the drug-induced effects on the phosphorylation of endogenous substrate proteins by particulate PKC. Thus, treatment with LY 171555 decreased the phosphorylation of the 17K and 21K proteins in the presence of low concentrations of added calcium, and this effect was abolished by prior treatment with reserpine (Fig. 2, upper panel). The densitometric areas for the 17K protein phosphorylated in the presence of 1, 5 or 15 μM calcium were 43, 85 and 105% of control activity, respectively, in the LY 171555 treated group, and 137, 127 and 111% of control activity, respectively, in the LY 171555 and reserpine pretreated group. The phosphorylation of the 21K protein in the presence of 1, 5 or 15 μM added calcium was 45, 77 and 88% of control activity, respectively, in the LY 171555 treated group, and 150, 191 and 175% respectively, in the LY 171555 and reserpine pretreated group. Similar effects were seen with SKF 38393 (Fig. 2, lower panel), where the decrease in the endogenous phosphorylation was reversed by pretreatment with reserpine. For example, the phosphorylation of the 17K protein in the presence of 5 or 15 μM added calcium was 30 and 48% of control activity, respectively, in the SKF 38393 treated group, and 51 and 90%, respectively, in the SKF 38393 and reserpine pretreated group. The phosphorylation of the 21K protein in the presence of 5 or 15 μM calcium was 32 and 46%, respectively, in the SKF 38393 treated group, and 52 and 60%, respectively, in the SKF 38393 and reserpine pretreated group.

Besides the DA agonists, the effect of GBL on endogenous phosphorylation by PKC was also reversed by reserpine pretreatment (Fig. 3). For

Table 4. Effects of reserpine pretreatment for 20 min on the K_m of PKC for calcium

Treatment	Soluble PKC		Particulate PKC	
	K_m (μM)	% Control	K_m (μM)	% Control
Control	5.5 ± 1.1	100	17.0 ± 0.9	100
Res	13.9 ± 1.6*	251	7.8 ± 1.1†	46
LY	0.7 ± 0.2*	13	36.5 ± 2.8†	215
Res + LY	1.1 ± 0.1*	20	26.7 ± 2.2*	157
Sul	12.4 ± 1.1*	225	7.8 ± 0.2†	46
Res + Sul	89.7 ± 3.4†	1630	1.8 ± 0.4†	10

Rats were injected with reserpine (5 mg/kg) with and without LY 171555 (1 mg/kg) or sulpiride (100 mg/kg) prior to being killed. The striata were fractionated into soluble and particulate fractions, each of which was analyzed for PKC activity as described in the text. Abbreviations: Res, reserpine; LY, LY 171555; and Sul, sulpiride. Results are expressed as the mean ± SE (N = 4) for the K_m for calcium and as the percent of control activity.

* $P < 0.05$ vs control.

† $P < 0.01$ vs control.

Table 5. Effects of reserpine pretreatment for 3 hr on PKC activity

Treatment	PKC activity (% of control)			
	Soluble		Particulate	
	K_m	V_{max}	K_m	V_{max}
(1) Res	113 ± 7	74 ± 4*	100 ± 9	82 ± 4*
(2) SKF	51 ± 5†	93 ± 6	360 ± 33†	93 ± 6
SKF + Res	105 ± 9	92 ± 7	130 ± 27	105 ± 7
(3) LY	53 ± 3†	98 ± 6	314 ± 9†	117 ± 6
LY + Res	99 ± 2	100 ± 4	139 ± 17	94 ± 5
(4) Sch	231 ± 21*	107 ± 2	24 ± 5†	96 ± 7
Sch + Res	101 ± 12	94 ± 6	104 ± 11	94 ± 13
(5) Sul	157 ± 10*	113 ± 10	53 ± 5*	113 ± 15
Sul + Res	94 ± 10	108 ± 5	112 ± 10	115 ± 14
(6) GBL	60 ± 5†	109 ± 8	224 ± 28*	91 ± 14
GBL + Res	101 ± 11	95 ± 3	108 ± 9	108 ± 16

Rats were pretreated with reserpine (5 mg/kg) 3 hr prior to injection of the DA-acting drugs. See Methods for dose of these drugs. Thirty minutes later, the rats were killed, and the striata were analyzed for soluble and particulate PKC activities as described in the text. Results are expressed as percent of the control K_m and V_{max} (mean ± SE, N = 4). See Table 1 for control values. GBL = α-butyrolactone.

* $P < 0.05$ vs control.

† $P < 0.01$ vs control.

example, the phosphorylation of the 17K protein in the presence of 5 and 15 μM calcium was 36 and 86% of control activity, respectively, in the GBL treated group, and 63 and 98%, respectively, in the GBL and reserpine pretreated group. Likewise, the phosphorylation of the 21K protein in the presence of 5 and 15 μM calcium was 13 and 79% of control activity, respectively, in the GBL treated group, and 92 and 93% respectively, in the GBL and reserpine pretreated group.

The effects of the DA antagonists on endogenous phosphorylation were also prevented by pretreatment with reserpine. Thus, the phosphorylation of the 17K and 21K proteins by particulate PKC were increased by 56 and 83%, respectively, in the sulpiride treated group, while they were increased by 11 and 11%, respectively, in the sulpiride and reserpine pretreated group. Similar effects were seen with Sch 23390 (data not shown).

Treatment with DA-acting drugs has been shown previously to alter calmodulin-dependent protein kinase activity in the particulate fraction in a manner opposite to that of particulate PKC activity [2]. These effects on calmodulin-dependent kinase activity were also prevented by pretreatment with reserpine. Thus, treatment with the DA agonists (apomorphine, SKF 38393 and LY 171555) or GBL resulted in a decrease in the K_m for calcium, and these effects were not seen in rats pretreated with reserpine (Table 6). On the other hand, treatment with the DA antagonists (Sch 23390 and sulpiride) resulted in an increase in the K_m for calcium, and these effects were also prevented by the reserpine pretreatment (Table 6).

The effects on calmoduline-dependent protein kinase activity were also reflected in the phosphorylation of endogenous substrate proteins (Fig. 4), which were altered at low concentrations of added calcium. Thus, at 1 and 5 μm calcium, the phosphorylation of the 51K protein was 175 and 117% of control activity, respectively, in the LY 171555

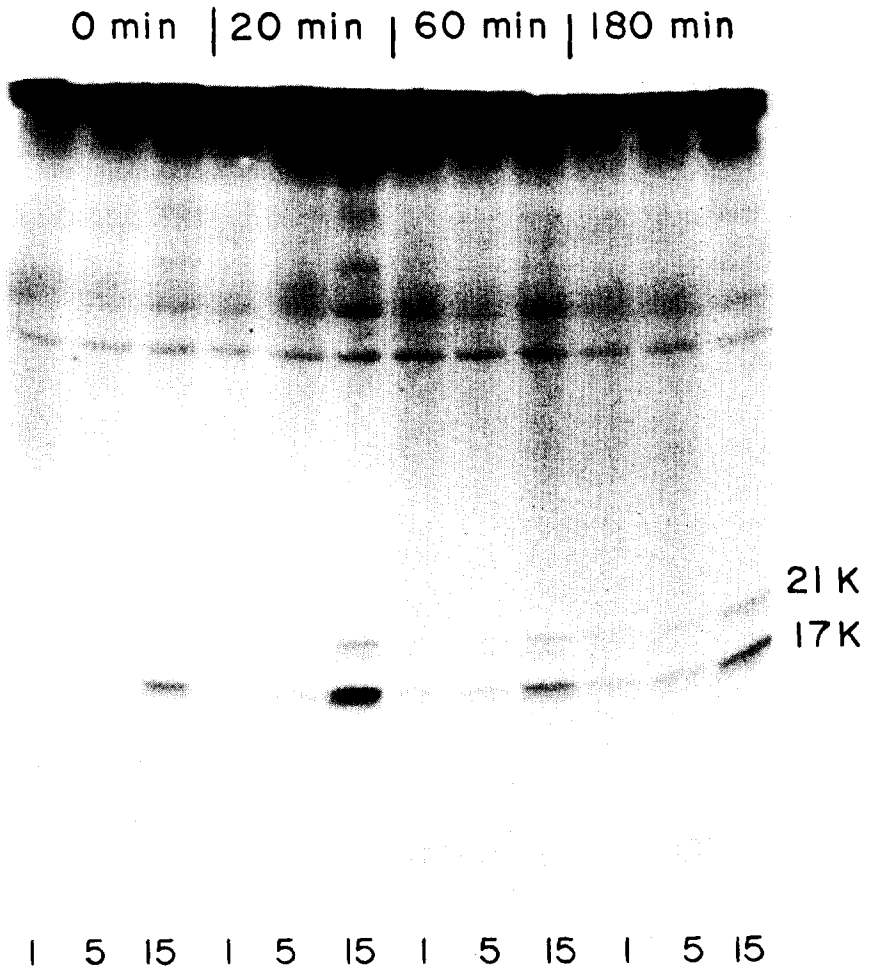

Fig. 1. Changes in endogenous phosphorylation by particulate PKC at various times after reserpine treatment. Reserpine (5 mg/kg) was injected 20, 60 or 180 min prior to killing the rats, and striata were analyzed for endogenous phosphorylating activity in the presence of lipids and 1, 5 or 15 μM calcium, using SDS–PAGE and autoradiography as described in the text. The numbers on the right indicate the $M_r \times 10^{-3}$.

treated group, 84 and 87%, respectively, in the LY 171555 and reserpine pretreated group, 138 and 139%, respectively, in the SKF 38393 treated group, and 100 and 78%, respectively, in the SKF 38393 and reserpine pretreated group. Likewise, the phosphorylation of the 62K protein in the presence of 1 and 5 μM calcium was 133 and 118% of control activity, respectively, in the LY 171555 treated group, 92 and 100%, respectively, in the LY 171555 and reserpine treated group, 150 and 106%, respectively, in the SKF 38393 treated group, and 100 and 71%, respectively, in the SKF 38393 and reserpine pretreated group.

The changes in endogenous phosphorylation by calmodulin-dependent protein kinase induced by GBL were also prevented by the reserpine pretreatment (Fig. 5). This effect was most evident at 5 μM calcium. Thus, the phosphorylation of the 51K protein in the presence of 5 and 15 μM calcium was 200 and 80% of control activity, respectively, in the GBL treated group, and 75 and 73%, respectively, in the GBL and reserpine pretreated group. The phosphorylation of the 62K protein in the presence of 5 and 15 μM calcium was 375 and 75%, respectively, of control activity in the GBL treated group, and 75 and 89%, respectively in the GBL and reserpine pretreated group.

DISCUSSION

A modulatory role of protein kinase C has been implicated in the secretion of hormones and neurotransmitters [1]. Its involvement in the release of dopamine in the nigrostriatal pathway has been suggested, based partly on the finding that there was a correlation between the ability of drugs to alter the inpulse-induced release of DA and their ability to affect PKC activity in the striatum [2]. In this study, it was found that the effects of these DA-acting drugs on PKC activity can be prevented by pretreatments which abolish the electrically evoked release of DA. This indicates that an intact releasable pool of DA is required for the changes in PKC activity. It seems to suggest that these changes may occur at the presynaptic DA terminal rather than on postsynaptic striatal neurons since prior depletion of presynaptic

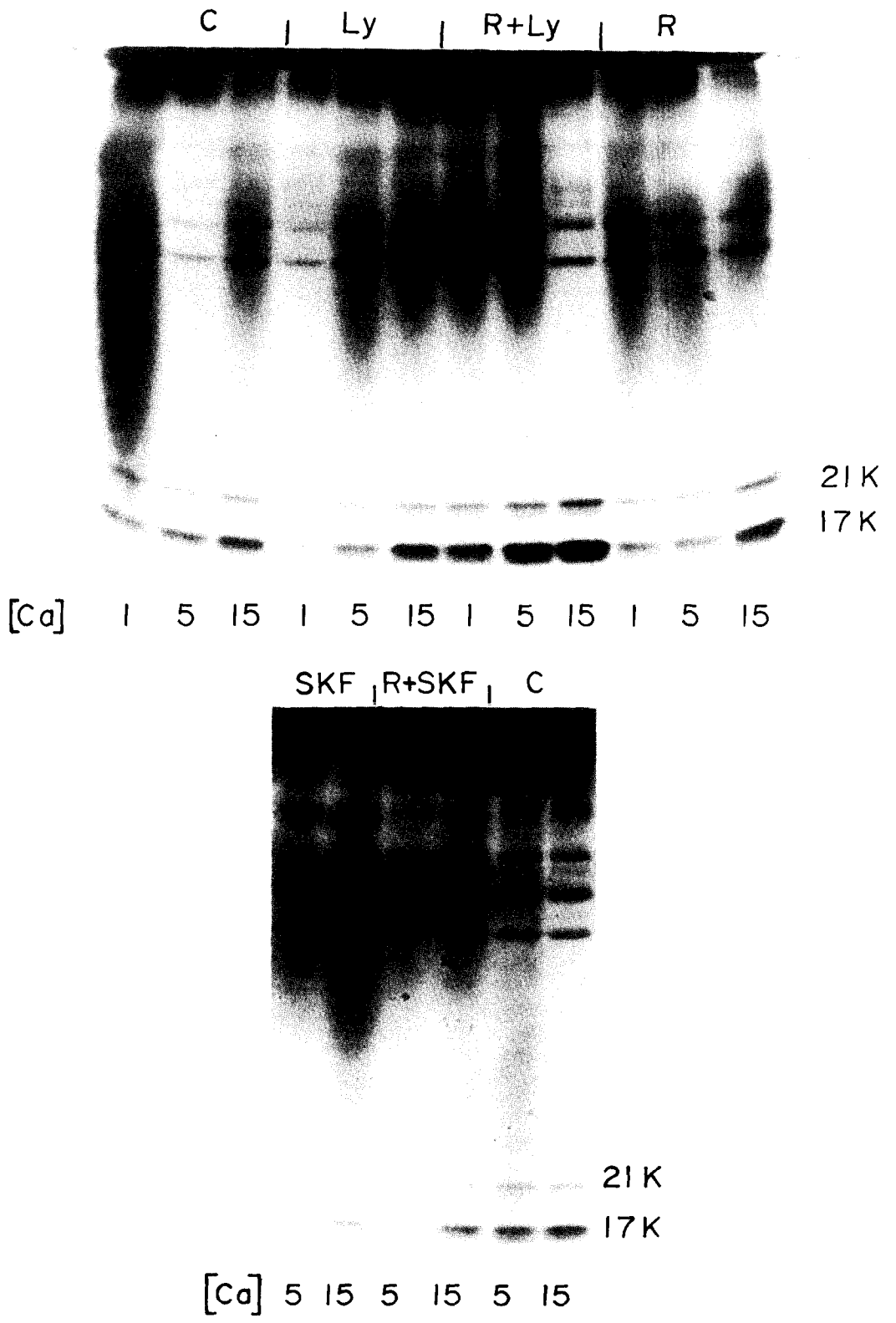

Fig. 2. Effects of reserpine pretreatment on changes in particulate PKC activity induced by the DA agonists (upper panel) LY 171555 and (lower panel) SKF 38393. Rats were pretreated with reserpine (R) (5 mg/kg) 3 hr prior to injection of LY 171555 (1 mg/kg) (R + Ly) or SKF 38393 (30 mg/kg) (R + SKF). The rats were killed 30 min later, and the striata were analyzed for endogenous phosphorylation in the presence of lipids and 1, 5 and 15 μM calcium as described in the text. Results from control rats (C), and rats treated with LY 171555 alone (Ly) or SKF 38393 alone (SKF) are also presented. The numbers on the right indicate $M_r \times 10^{-3}$.

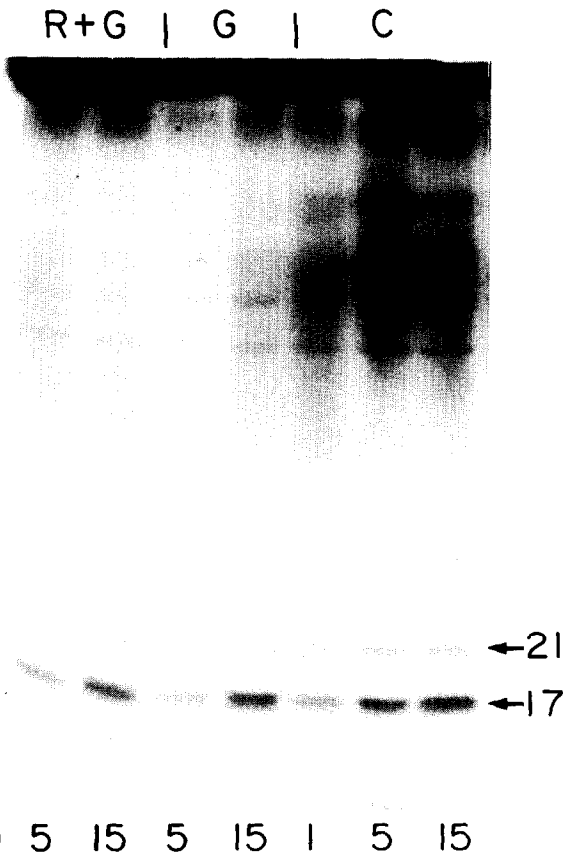

Fig. 3. Effects of reserpine pretreatment on changes in particulate PKC activity induced by GBL. Rats were pretreated with reserpine (5 mg/kg) 3 hr prior to injection with GBL (750 mg/kg) R + G). Striata were analyzed for endogenous phosphorylating activity in the presence of lipids and 1, 5 or 15 μM calcium as described in the text. Results of control rats (C) and rats treated with GBL alone (G) are also presented. The numbers on the right indicate the $M_r \times 10^{-3}$.

Table 6. Effects of reserpine pretreatment on calmodulin-dependent protein kinase activity

	Calmodulin kinase activity			
Treatment	K_m (μm)	% Control	V_{max} (pmol/min/mg tissue)	% Control
(1) None	2.98 ± 0.16	100	13.87 ± 0.55	100
(2) Res	2.11 ± 0.25	71	10.30 ± 0.50*	74
(3) Apo	0.58 ± 0.18†	19	16.23 ± 0.52	117
Apo + Res	3.49 ± 0.88	117	16.78 ± 0.88	121
(4) SKF	1.18 ± 0.24†	40	14.93 ± 0.76	108
SKF + Res	3.62 ± 0.38	121	17.75 ± 1.47	128
(5) LY	0.77 ± 0.03†	26	14.58 ± 0.03	105
LY + Res	3.55 ± 0.16	119	14.53 ± 0.12	105
(6) Sch	4.18 ± 0.19*	140	16.83 ± 0.91	121
Sch + Res	2.98 ± 0.16	100	15.91 ± 2.11	115
(7) Sul	5.16 ± 0.52*	173	15.53 ± 0.97	112
Sul + Res	2.58 ± 0.26	89	16.80 ± 0.83	121
(8) GBL	1.36 ± 0.24*	46	12.19 ± 0.46	88
GBL + Res	4.08 ± 0.61	137	12.84 ± 0.35	93

Rats were pretreated with reserpine (5 mg/kg) 3 hr prior to injection of the DA-acting drugs. See Methods for doses of these drugs. The striata were analyzed for calmodulin-dependent kinase activity in the particulate fraction as described in the text. Apo = apomorphine. Results are expressed as mean ± SE (N = 4) for K_m and V_{max} and as percent of control activity.

* $P < 0.05$ vs controls.

† $P < 0.01$ vs control.

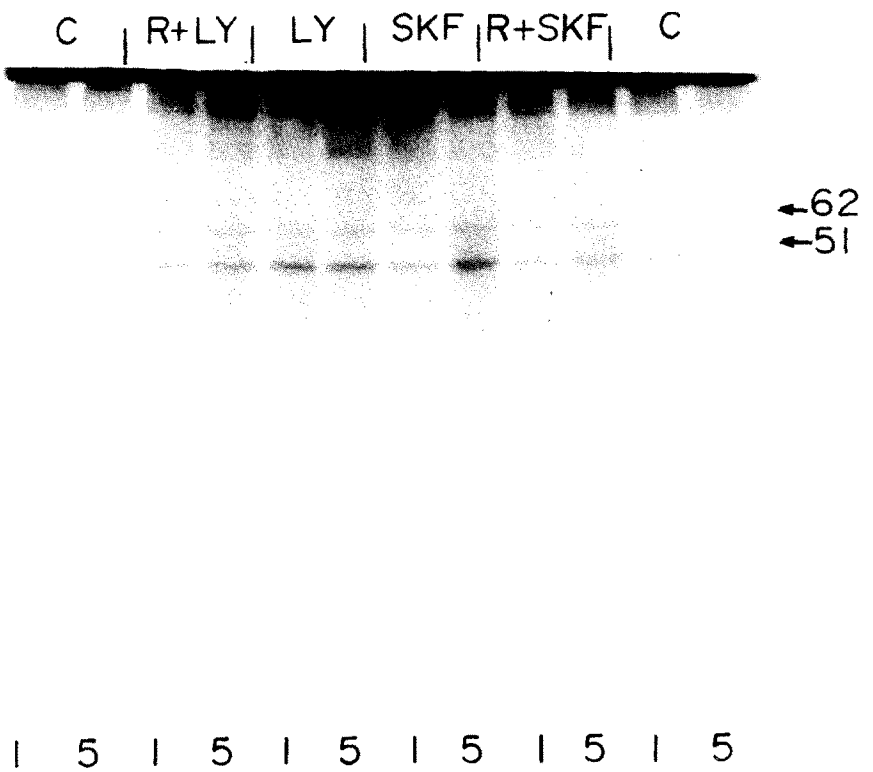

Fig. 4. Effects of reserpine pretreatment on changes in calmodulin-dependent protein kinase activity induced by LY 171555 and SKF 38393. Rats were pretreated with reserpine (5 mg/kg) 3 hr prior to injection of LY 171555 (1 mg/kg) (R + LY) or SKF 38393 (30 mg/kg) (R + SKF). The rats were killed 30 min later, and the striata were dissected. The particulate fractions were analyzed for endogenous phosphorylating activity in the presence of calmodulin and 1 or 5 μM calcium as described in the text. Results from control rats (C), and rats treated with LY 171555 alone (LY) or SKF 38393 alone (SKF) are also presented. The numbers on the right indicate the $M_r \times 10^{-3}$.

DA stores should have no effect on postsynaptic PKC activity, if these DA agonists and antagonists have acted directly at the postynaptic receptor site. On the other hand, there are complex interactions between endogenous DA and DA drugs at the postsynaptic receptor [6], such that it is possible that the changes in PKC activity may be occurring postsynaptically. Furthermore, even if the changes in PKC activity are at the presynaptic DA terminal, the postynaptic DA receptor can be involved indirectly in altering PKC activity and DA release by altering the firing rate of DA neurons via the efferent feedback loop. The cellular pathways by which the DA-acting drugs alter PKC activity have yet to be determined.

The preventive effects of reserpine and α-MT on the changes in PKC activity induced by the DA-acting drugs are due to a specific diminution of DA: (1) α-MT is a specific inhibitor of DA synthesis even though reserpine can also decrease serotoning at high doses [5]; (2) higher doses of α-MT which reduced DA levels more, were more effective than the lower doses, which reduced DA levels less; and (3) reserpine prevented the effects of sulpiride on PKC activity at a time when the decrease of DA should be maximal (1–3 hr) and not at 20 min when substantial amounts of DA are still present [7].

The mechanism underlying the differential changes in PKC activity with time of reserpine treatment is not known. It may be related to changes in synthesis rates induced by reserpine: synthesis is decreased at 40 min and increased maximally at 3 hr [7]. This is, however, improbable, in lieu of the fact that acute treatments (20–30 min) with reserpine or neuroleptics resulted in similar changes in PKC activity (decrease in the soluble and increase in the particulate PKC activity) but different changes in synthesis rates (reserpine decreases and neuroleptics increase synthesis). Thus, it is unlikely that changes in DA synthesis can account for the changes in PKC activity following the administration of these drugs. The question as to whether PKC plays a role in modulating DA synthesis has been examined [8, 9].

The initial effects of reserpine on PKC activity are qualitatively similar to those seen with the DA antagonists. They may be due to an immediate decrease in DA levels at the autoreceptor site since the effects were abolished by administration of the D_2 agonist, LY 171555, and potentiated by the D_2 antagonist, sulpiride. The additive nature of the effects of reserpine and sulpiride indicates that they act via different mechanisms. Thus, while reserpine may exert its effects on PKC activity by disinhibiting the autoreceptor (via a decrease in DA release), sulpiride may additionally stimulate PKC activity by increasing the firing rate of the DAN neuron through

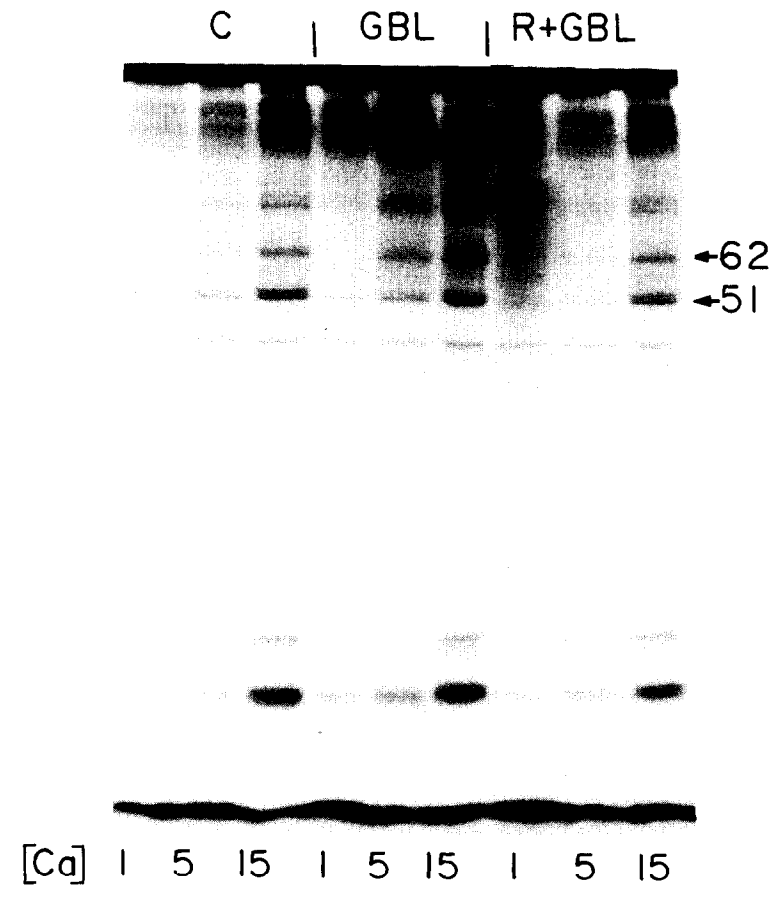

Fig. 5. Effects of reserpine pretreatment on changes in calmodulin-dependent protein kinase activity induced by GBL. Rats were pretreated with reserpine (5 mg/kg) 3 hr prior to injection with GBL (750 mg/kg) (R + GBL). The rats were killed 30 min later, and the striata were dissected. The particulate fractions were analyzed for endogenous phosphorylating activity in the presence of calmodulin and 1, 5 or 15 μM calcium as described in the text. Results of control rats (C) and rats injected with GBL alone (GBL) are also presented. The numbers on the right indicate the $M_r \times 10^{-3}$.

the efferent feedback loop. Sulpiride can affect DA release by these dual mechanisms.

In contrast to the initial effects of reserpine, a small but significant decrease in the V_{max} for calcium in both the soluble and particulate fractions was observed 1–3 hr after treatment. Similar effects were seen with α-MT. With this longer pretreatment interval, the effects of the DA-acting drugs on PKC activity were abolished. The molecular mechanism for this attenuation is not known. It cannot be explained by a decrease in DA levels at the autoreceptor site, since the depleting drugs abolished the effects not only of the DA agonists but also of the DA antagonists, GBL and SKF 38393, which do not bind to the autoreceptor site. Furthermore, it cannot be explained by a reserpine-induced increase in impulse flow [10] since reserpine attenuated the effects of the DA antagonists, which also increase the firing rate of the DA neurons. It is therefore unlikely that a change in depolarization underlies the protective effects of the DA-depleting drugs on PKC activity.

A possible mechanism involves the decrease in ATP concurrent with the loss of DA. Reserpine treatment has been shown to cause a large decrease in ATP levels in chromaffin vesicles [11]. Since ATP is required for the synthesis of the poly-phosphatidylinositides (poly-PIs) [12], a reserpine-induced decrease in ATP could lead to a decrease in the level of the poly-PIs, and consequently a decrease in the level of their breakdown product, diacylglycerol. The latter serves as the physiological regulator of PKC activity [13], and a lack of diacylglycerol will result in a de-regulation of PKC activity in spite of receptor activation. This may explain why reserpine can attenuate the effects of DA-acting drugs, regardless of whether they are direct DA antagonists or antagonists or indirect DA-acting agents (GBL), as would be the case if PKC activity has been de-regulated. This model is compatible with the observations that the DA-acting drugs altered PKC activity in a manner similar to that seen with diacylglycerol, viz. by decreasing the K_m for calcium. This model assumes that the DA-acting drugs can alter the metabolism of the poly-PIs and that reserpine treatment decreases the ATP level sufficiently to increase the synthesis of the poly-PIs. Work is in progress to examine the validity of these assumptions.

Injection of the DA-acting drugs also affected

calmodulin-dependent protein kinase activity in the particulate fraction but the direction of this effect was opposite to that of particulate PKC activity [2]. In this study, it was found that the depletion of DA also prevented the changes in calmodulin-dependent protein kinase activity. The relationship between the two protein kinases needs to be clarified.

It has been generally assumed that protein kinases are involved in the modulation of the evoked release of neurotransmitters from synaptic vesibles. The present observation that reserpine, which depletes the vesicular pool of DA, was as effective as α-MT, which depletes the newly synthesized DA pool as well as the vesicular pool, in preventing the effects of the DA-acting drugs on PKC activity suggests that an intact vesicular pool is essential for the changes in PKC activity induced by these drugs. This is compatible with the assumed role of PKC in the exocytotic release of DA [14, 15]. This involvement may be at the level of modulation rather than mediation since treatments which abolish PKC-induced release have no effect on calcium-dependent release [16, 17] and phorbol esters are ineffective in enhancing release in the absence of calcium [18]. The mechanism by which PKC modulates release is not known.

In summary, this study shows that agents which lowered endogenous DA also prevented the changes in PKC and calmodulin-dependent protein kinase activities induced by DA agonists and antagonists and by GBL. These results suggest that an intact releasable pool of DA is essential for the effects of the DA-acting drugs and are compatible with the hypothesis that these protein kinases are involved in the modulation of exocytotic release of DA *in vivo*.

Acknowledgements—The author would like to thank Drs William Rosen and Robert Patrick for reviewing the manuscript, and Suzette Walpole for excellent technical assistance. This study is supported by the Institute of Mental Health, a division of Mental Health, Retardation and Hospitals of Rhode Island.

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