British Journal of Pharmacology (1997) 122, 1561–1566

Modulation of dopamine release from rat striatum by protein kinase C: interaction with presynaptic D_2-dopamine-autoreceptors

[1]L. Iannazzo, S. Sathananthan & H. Majewski

Prince Henry's Institute of Medical Research, P.O. Box 5152, Clayton, Victoria, Australia 3168

1 Interactions between dopamine receptors and protein kinase C (PKC) have been proposed from biochemical studies. The aim of the present study was to investigate the hypothesis that there is an interaction between protein kinase C and inhibitory D_2-dopamine receptors in the modulation of stimulation-induced (S-I) dopamine release from rat striatal slices incubated with [^{3}H]-dopamine. Dopamine release can be modulated by protein kinase C and inhibitory presynaptic D_2 receptors since phorbol dibutyrate (PDB) and (−)-sulpiride, respectively, elevated S-I dopamine release.

2 The protein kinase C inhibitors polymyxin B (21 μM) and chelerythrine (3 μM) had no effect on stimulation-induced (S-I) dopamine release. However, when presynaptic dopamine D_2 receptors were blocked by sulpiride (1 μM), an inhibitory effect of both PKC inhibitors on S-I dopamine release was revealed. Thus, sulpiride unmasks an endogenous PKC effect on dopamine release which suggests that presynaptic D_2 receptors normally suppress endogenous PKC activity. This is supported by results in striatal slices which were pretreated with PDB to down-regulate PKC. In this case the facilitatory effect of sulpiride was completely abolished.

3 The inhibitory effect of the dopamine D_2/D_3 agonist quinpirole on S-I dopamine release was partially attenuated by PKC down-regulation. Since the effect of sulpiride was completely abolished under the same conditions, this suggests that exogenous agonists may target a PKC-dependent as well as a PKC-independent pathway. The inhibitory effect of apomorphine was not affected by either polymyxin B or PKC down-regulation, suggesting that it operated exclusively through a PKC-independent mechanism.

4 These results suggest that there are at least two pathways involved in the inhibition of dopamine release through dopamine receptors. One pathway involves dopamine receptor suppression of protein kinase C activity, perhaps through inhibition of phospholipase C activity and this is preferentially utilized by neuronally-released dopamine. The other pathway which seems to be utilized by exogenous agonists does not involve PKC.

Keywords: Protein kinase C; dopamine release; phorbol esters; polymyxin B; sulpiride; apomorphine; presynaptic receptors; D_2-dopamine receptors

Introduction

Protein kinase C (PKC) is a family of intracellular enzymes which can be activated by second messengers generated by membrane phospholipid breakdown initiated by phospholipases. The principal physiological activator of PKC is diacylglycerol which is produced by phospholipase C or alternately through phospholipase D via phosphatidic acid (Nishizuka, 1989; 1992). Phorbol esters mimic the actions of diacylglycerol on PKC and markedly enhance the action-potential evoked release of dopamine: (cultured foetal neurones, Zurgil *et al*., 1986; rat amygdala, Versteeg & Ulenkate, 1987; rabbit striatum, Cubeddu *et al*., 1991; rabbit prefrontal cortex, Talmaciu *et al*., 1989; rat striatal synaptosomes, Chandler & Leslie, 1989; rat brain synaptosomes, Nichols *et al*., 1987; rat mesencephalic cells in primary culture, Brouard *et al*., 1994; rat cortex, Kotsonis & Majewski, 1996). The PKC inhibitor polymyxin B inhibited stimulation-induced dopamine release from rat amygdala (Versteeg & Ulenkate, 1987), as well as in a microdialysis study in rat striatum *in vivo* (Xu *et al*., 1990), which suggests that action potential evoked dopamine release is modulated by PKC under some physiological conditions.

Automodulation of dopamine release occurs through inhibitory presynaptic D_2-dopamine receptors (Starke *et al*., 1989) and it is possible that these receptors may interact with the phospholipase C/PKC pathway at several levels. For example, in rat striatum D_2-receptor activation inhibits receptor stimulated inositol phosphate accumulation (Pizzi *et al*., 1987), although other workers have not found an effect in the same tissue (Kelly *et al*., 1988). It has also been shown that activation of dopamine D_2-receptors inhibits PKC activity in striatal synaptoneurosomes (Giambalvo & Wagner, 1994) and PKC reduces high affinity D_2-receptor agonist binding *in vitro* (Rogue *et al*., 1990). Furthermore PKC can alter dopamine D_2 receptor signal transduction coupling (Liu *et al*., 1992; Di Marzo *et al*., 1993) and PKC mediated phosphorylation can inhibit the neuronal uptake of dopamine in striatal synaptosomes (Copeland *et al*., 1996).

Given the above evidence, it was the hypothesis of the present study that PKC may be involved either directly or indirectly in the signal transduction pathway of D_2-autoreceptors in striatum. This was investigated by monitoring stimulation-induced dopamine release in slices of the rat striatum which were incubated with [^{3}H]-dopamine.

Methods

Male Sprague Dawley rats weighing 150–250 g were killed by guillotine. The brains were rapidly excised and transferred to ice-cold physiological salt solution (PSS). A coronal cut of the brain was made at the level of the hypothalamus and the rostral part of the brain was mounted on a tissue slicer. The first coronal slice was made 3.5 mm caudal to the anterior-most part of the brain. Further slices (400 μm thick) were obtained. Only 2 slices were used from each brain. The corpus striatum was identified in each slice and cut out for use.

[1] Author for correspondence.

Experimental protocol

After dissection, the striatal slices were incubated for 30 min in physiological salt solution (2 ml) containing [^{3}H]-dopamine (5 μCi ml^{-1}, 0.4 μM) maintained at 37°C and bubbled continuously with a mixture of 5% CO_2 and 95% O_2 similar to Cubeddu *et al*. (1991). After incubation, each slice was transferred to a superfusion chamber where it was placed in a plastic holder between a pair of parallel platinum electrodes 5 mm apart. Slices were maintained at 37°C and superfused with physiological salt solution at 0.8 ml min^{-1}.

The striatal slices were subjected to 3 periods of electrical field stimulation (priming, S_1, and S_2) with monophasic square wave pulses (field strength 7.5 V cm^{-1} per flow cell), 2 ms duration, at a frequency of 3 Hz. Priming stimulation was for 30 s while S_1 and S_2 were for 120 s. For experiments that involved other frequencies of stimulation, priming and S_1 were kept constant (i.e. 3 Hz) while S_2 was changed to 0.3 or 10 Hz. Beginning of superfusion was taken as 0 min. Priming stimulation occurred at 30 min, S_1 at 60 min and S_2 at 104 min. Drugs were introduced into the superfusion solution 20 min before S_2 (at 84 min) (except for polymyxin B which was added 40 min before S_2). The superfusate was collected in 2 min fractions starting 6 min before S_1 and continuing to 20 min after S_2. The dopamine neuronal uptake blocker, nomifensine (1 μM) was present in the superfusion solution from immediately after the [^{3}H]-dopamine incubation.

At the end of superfusion, slices were dissolved in 1 ml Soluene for at least 12 h. The radioactive content in superfusate fractions and tissue were determined by liquid scintillation counting and corrected for counting efficiency by automatic external standardization.

Long-term treatment with phorbol 12, 13-dibutyrate

Rat striatal brain slices were prepared as described previously. The slices were placed in 50 ml modified PSS containing 0.1 mM Ca^{2+}, dextran (50 g l^{-1}, average MW = 70,000) and either phorbol 12, 13-dibutyrate (1 μM) or vehicle (dimethyl sulphoxide, DMSO 0.06% v:v) in an open dish and maintained at 32°C in a tissue culture incubator for 20 h. The dextran was used to maintain oncotic pressure and the Ca^{2+} was lower than normal PSS to minimize possible toxic effects of Ca^{2+} over the 20 h period. The atmosphere of the incubator was a mixture of 5% CO_2 and 95% O_2. At the end of the 20 h incubation, the brain slices were removed from the culture medium and washed in 100 ml PSS. The slices were then incubated with [^{3}H]-dopamine in normal PSS as previously described and then followed the identical protocol as described for acute experiments.

Calculations

The fractional outflow of radioactivity was calculated as the amount of radioactivity in each 2 min sample divided by the total tissue radioactivity at the beginning of the respective sample collection. The mean resting (spontaneous outflow) was taken as the mean of the fractional outflow of the two samples before each stimulation. The fractional stimulation-induced outflow of radioactivity was calculated as the sum of the fractional outflow in the eight 2 min periods from the start of stimulation minus eight times the mean fractional resting outflow. The fractional stimulation-induced outflow of radioactivity evoked by S_2 was expressed as a percentage of that evoked by S_1. There were minor changes when the stimulation was at 0.3 Hz for 400 s in that the stimulation-induced component was taken as the mean of 10 samples following the commencement of stimulation.

Statistics

Results are expressed as mean ± s.e.mean and n within each column indicates the number of experiments. Significance of differences between means was calculated with Student's unpaired t test. Where multiple results were compared to a single control Dunnett's test after one way analysis of variance was used. A probability of type 1 error of less than 5% ($P<0.05$) was taken to indicate statistical significance.

Materials

The physiological salt solution (PSS) contained (mM): NaCl 118.0, KCl 4.7, KH_2PO_4 1.03, $NaHCO_3$ 25.0, D-(+)-glucose 11.1, $CaCl_2$ 1.3, $MgSO_4$ 1.2, ascorbic acid 0.14, disodium EDTA 0.067, pH = 7.4. Tetrodotoxin, 4β-phorbol 12, 13-dibutyrate (PDB), polymyxin B sulphate, **S**(−)-sulpiride, chelerythrine chloride and **R**(−)-apomorphine hydrochloride were obtained from Sigma (St-Louis, U.S.A.). Nomifensine maleate and (−)-quinpirole hydrochloride were purchased from Research Biochemicals Inc (Natick, U.S.A.). Soluene-100 and Picofluor 40 were obtained from Packard Instruments (Downers Grove, U.S.A.). [2,5,6-^{3}H]-dopamine hydrochloride (specific activity 13 Ci mmol^{-1}) was purchased from Amersham International (England). Stock solutions of PDB were made up in dimethyl sulphoxide and stored at −20°C. Other drugs were prepared freshly in physiological salt solution.

Results

Dopamine release from striatal slices

Electrical stimulation of the striatal slices, which had been incubated with [^{3}H]-dopamine, evoked a stimulation-induced outflow of radioactivity. The fractional S-I outflow of radioactivity in the second stimulation was not observed in the absence of extracellular Ca^{2+} and was virtually abolished by tetrodotoxin (0.3 μM). (S_2 as a % of $S_1 = 85 \pm 1$, $n = 14$ for control, 4 ± 1*, $n = 6$ for Ca^{2+}-free and 14 ± 3*, $n = 4$ for tetrodotoxin; *significantly different from control $P<0.05$). None of the drugs affected the spontaneous outflow of radioactivity.

Modulation of dopamine release by PKC

The PKC activator phorbol dibutyrate (PDB, 0.03 − 1 μM) concentration-dependently enhanced the fraction S-I outflow of radioactivity (Figure 1). This effect was blocked by the PKC inhibitor polymyxin B (21 μM). A higher concentration of polymyxin B (70 μM) by itself inhibited the fractional S-I outflow and also blocked the facilitatory effect of PDB (Figure 1). The PKC inhibitor chelerythrine (3 μM) also inhibited the facilitatory effect of PDB without affecting the fractional S-I outflow by itself (Figure 2). None of the drugs affected the spontaneous outflow of radioactivity except for polymyxin B (70 μM) which significantly enhanced the spontaneous outflow to 128 ± 8% of control and the combination PDB (1 μM) and polymyxin B (70 μM) which elevated the spontaneous outflow to 210 ± 11% of control. These values were significantly different from control ($P<0.05$, Dunnett's test).

Presynaptic dopamine receptor modulation of dopamine release

The dopamine receptor agonist apomorphine (0.1 and 0.3 μM) concentration-dependently decreased the fractional S-I outflow of radioactivity (Figure 4) as did the D_2-selective agonist quinpirole (0.3 μM) (Figure 4). The D_2-selective dopamine receptor blocking drug (−)-sulpiride (0.1 − 10 μM) concentration-dependently enhanced the fractional S-I outflow of dopamine (S_2 as a % of $S_1 = 85 \pm 1$, $n = 14$ for control, 166 ± 3*, $n = 3$, sulpiride 0.1 μM; 196 ± 6*, $n = 5$, sulpiride 1.0 μM; 205 ± 10*, $n = 3$, sulpiride 10 μM; *significantly different from control $P<0.05$). These results indicate functional dopamine D_2 receptors in this system.

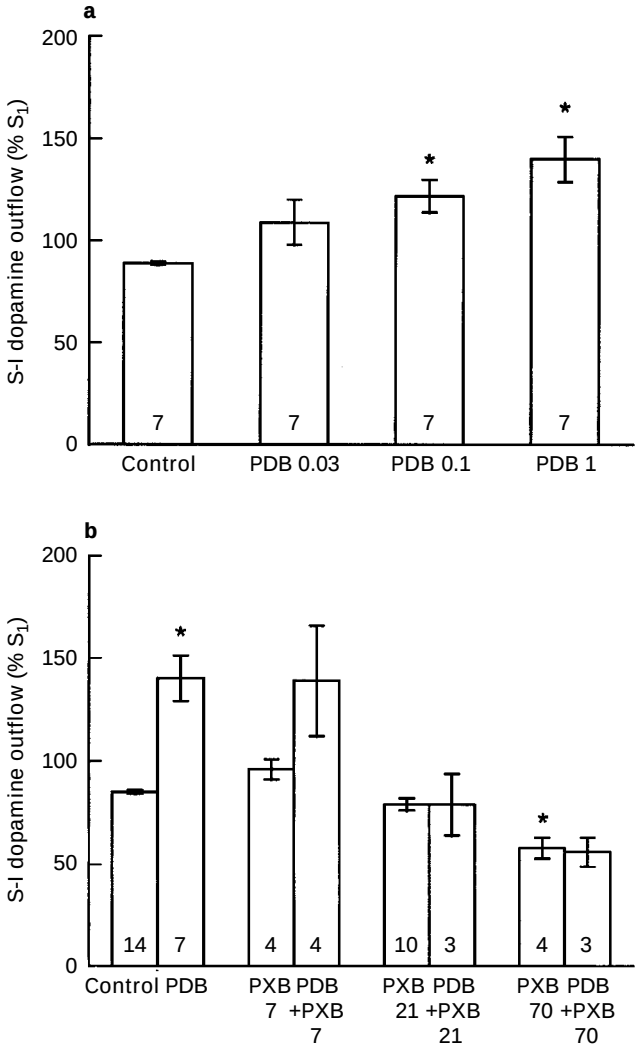

Figure 1 Effect of (a) phorbol dibutyrate (PDB, 0.03–1 μM) and (b) polymyxin B (PXB, 7–70 μM) on the fractional stimulation-induced (S-I) outflow of radioactivity (S-I dopamine outflow) from rat striatal slices pre-incubated with [^{3}H]-dopamine. There were two periods of test stimulation (S_1 and S_2) each at 3 Hz for 120 s. PDB and PXB were added at the concentrations indicated from 40 min before S_2. The fractional S-I outflow of radioactivity in S_2 is expressed as a percentage of that in S_1. The columns represent the means and the vertical lines the s.e.mean; the number of experiments is shown at the base of each column. *Represents significant effect of drug compared to control experiments in absence of drug ($P<0.05$).

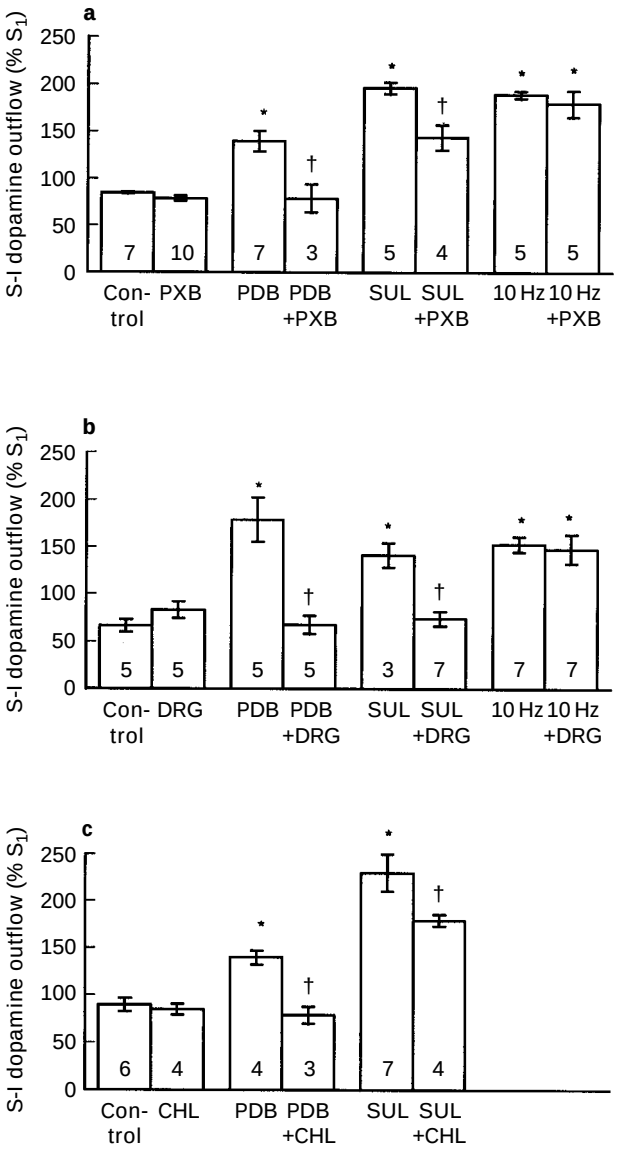

Figure 2 Effect of PKC inhibitors, (a) polymyxin B (PXB) and (c) chelerythrine (CHL), and (b) prolonged treatment with PDB (1 μM for 20 h, DRG) on sulpiride facilitation of the fractional stimulation-induced (S-I) outflow of radioactivity (S-I dopamine outflow) from rat striatal slices pre-incubated with [^{3}H]-dopamine. There were two periods of test stimulation (S_1 and S_2) the first at 3 Hz for 120 s and the second usually at 3 Hz but in some cases where indicated at 10 Hz. The fractional S-I outflow of radioactivity in S_2 is expressed as a percentage of that in S_1. Drugs (sulpiride, SUL 1 μM; polymyxin B, PXB 21 μM); chelerythrine, CHL 3 μM; phorbol dibutyrate, PDB 1 μM) were present for S_2. The columns represent the means and the vertical lines the s.e.mean, the number of experiments is at the base of each column. In (b) the striatal slices were incubated in either vehicle (DMSO) or PDB (1 μM) containing PSS for 20 h before being washed and incubated with [^{3}H]-dopamine. *Represents significant effect of drug compared to control experiments in absence of drug ($P<0.05$). †Represents a significant difference from respective experiment without PKC inhibitor or PDB treatment ($P<0.05$).

Effect of inhibition or downregulation of PKC on dopamine receptor modulation of dopamine release

In order to down-regulate PKC, striatal slices were treated in modified PSS medium containing 0.1 mM Ca^{2+}, dextran and either PDB (1 μM) or vehicle (DMSO) for 20 h before being incubated with [^{3}H]-dopamine. The vehicle-treated slices had a decreased fractional S-I outflow of radioactivity when compared to freshly excised cortical slices (compare vehicle-treated and untreated, Table 1) and an increased fractional resting outflow (Table 1). PDB treatment had similar effects to vehicle treatment (Table 1).

In rat cortical slices which were treated for 20 h with vehicle before incubation with [^{3}H]-dopamine, PDB enhanced the fractional S-I outflow of radioactivity (Figure 2). However, when the slices were treated for 20 h in PSS with PDB (1 μM), subsequent application of PDB, failed to enhance the fractional S-I outflow of radioactivity (Figure 2) indicating functional down-regulation of PKC.

The facilitation of the fractional S-I outflow of radioactivity by sulpiride was significantly inhibited by polymyxin B, chelerythrine and by PKC down-regulation when the stimulation was at 3 Hz for 2 min (Figure 2). Further, when the stimulation was at 0.3 Hz for 400 s the facilitatory effect of sulpiride was also decreased by chelerythrine (Figure 3). The preceding experiments were conducted in the presence of the dopamine uptake inhibitor nomifensene and when this was omitted then sulpiride no longer facilitated the S-I outflow (Figure 3). The

Table 1 The fractional resting and stimulation-induced outflow of radioactivity associated with the first stimulation period from rat striatal slices pre-incubated with [^{3}H]-dopamine

	R_1	S_1
Untreated ($n=201$)	0.0043 ± 0.0001	0.0731 ± 0.0002
Vehicle-treated ($n=36$)	0.0073 ± 0.0005*	0.0186 ± 0.0020*
PDB-treated ($n=42$)	0.0076 ± 0.0003*	0.0214 ± 0.0026*

There were several types of experiments. Firstly untreated freshly excised striatal slices, secondly striatal slices placed in an incubator for 20 h with either PDB (1 μM) or vehicle (DMSO) in the bathing solution before being taken for labelling with radioactive transmitter. R_1 represents the outflow of radioactivity min^{-1} immediately before the first stimulation period expressed as a fraction of tissue radioactivity. S_1 represents the total stimulation-induced portion of the radioactive outflow at S_1 (1 Hz for 60 s) expressed as a fraction of the tissue radioactivity. *Represents a significant difference from untreated slices, $P<0.05$.

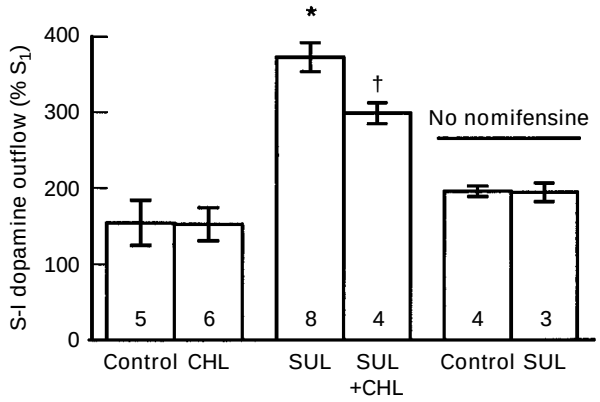

Figure 3 Effect of PKC inhibitor chelerythrine (3 μM) on sulpiride facilitation of the fractional stimulation-induced (S-I) outflow of radioactivity (S-I dopamine outflow) from rat striatal slices pre-incubated with [^{3}H]-dopamine. There were two periods of test stimulation (S_1 and S_2) the first at 3 Hz for 40 s and the second at 0.3 Hz for 400 s. In some experiments nomifensine was omitted from the physiological salt solution. The fractional S-I outflow of radioactivity in S_2 is expressed as a percentage of that in S_1. Drugs (sulpiride SUL 1 μM; chelerythrine CHL 3 μM) were present for S_2. The columns represent the means and the vertical lines the s.e.mean, the number of experiments is between 4–14 for each group. *Represents significant effect of drug compared to control experiments in absence of drug ($P<0.05$). †Represents a significant difference from respective experiment without chelerythrine ($P<0.05$).

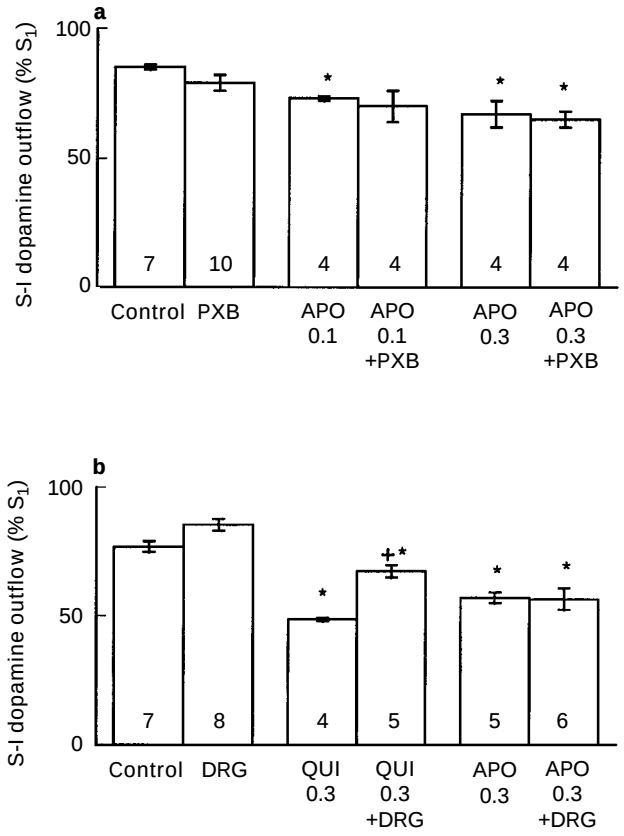

Figure 4 Effect of (a) PKC inhibitors and (b) prolonged treatment with PDB (1 μM for 20 h, DRG) on apomorphine (APO, 0.1 and 0.3 μM) and quinpirole (QUI, 0.3 μM) inhibition of the fractional stimulation-induced (S-I) outflow of radioactivity (S-I dopamine outflow) from rat striatal slices pre-incubated with [^{3}H]-dopamine. There were two periods of test stimulation (S_1 and S_2) at 3 Hz for 120 s. The fractional S-I outflow of radioactivity in S_2 is expressed as a percentage of that in S_1. Drugs (polymyxin B, PXB 21 μM; chelerythrine, CHL 3 μM; APO, QUI and phorbol dibutyrate, PDB 1 μM) were present for S_2. The columns represent the means and the vertical lines the s.e.mean, the number of experiments is at the base of each column. In (b) the striatal slices were incubated in either vehicle (DMSO) or PDB (1 μM) containing PSS for 20 h before being washed and incubated with [^{3}H]-dopamine. *Represents significant effect of drug compared to control experiments in absence of drug ($P<0.05$). †Represents a significant difference from respective experiment without PDB treatment ($P<0.05$).

inhibitory effect of the dopamine agonist quinpirole was partially attenuated in PKC down-regulated slices (Figure 4), whereas that of apomorphine was not affected by either polymyxin B or PKC down-regulation (Figure 4).

Discussion

Electrical stimulation of rat striatal brain slices, which were incubated with [^{3}H]-dopamine, resulted in the release of radioactivity which was frequency-dependent, abolished by tetrodotoxin and the absence of extracellular calcium indicating the neural release of dopamine. Stimulation-induced dopamine release was under inhibitory feedback modulation since the selective D_2-receptor blocking drug, sulpiride, concentration-dependently enhanced the S-I outflow of dopamine. This has been shown previously (Starke *et al*., 1989). Furthermore the dopamine receptor agonist apomorphine and the selective D_2/D_3-agonist quinpirole inhibited the S-I outflow of dopamine in accord with the existence of inhibitory presynaptic D_2 dopamine receptors (see Starke *et al*., 1989).

PKC modulates dopamine release in rat striatal slices since PDB concentration-dependently enhanced S-I dopamine release consistent with previous studies (see Introduction). This effect was blocked by the PKC inhibitors polymyxin B and the selective agent chelerythrine (Herbert *et al*., 1990). However, it is unlikely that there is an endogenous modulation of dopamine release through PKC, since the PKC inhibitors by themselves did not affect dopamine release in concentrations which completely blocked the effect of PDB. Higher concentrations of polymyxin B (70 μM) did inhibit S-I dopamine release, but this may be due to non-specific effects as previously shown at this concentration (Foucart *et al*., 1991). In contrast to these results, *in vivo* it has been suggested that PKC is tonically involved in the modulation of dopamine release in rat striatum by use of microdialysis techniques and 30 μM polymyxin B in the dialysis solution (Xu *et al*., 1990).

PKC can be down-regulated by prolonged exposure to phorbol esters and this in part is due to calpain-induced pro-

teolysis of PKC (e.g. Hong *et al*., 1995). In the present study we treated striatal slices with PDB for 20 h and found that subsequent application of PDB failed to enhance S-I dopamine release, which is indicative of a loss of PKC within the nerve terminals. Similar results have also been observed for noradrenergic nerves (Foucart *et al*., 1991; Kotsonis & Majewski, 1996). PDB treatment did not alter the S-I outflow of dopamine, reinforcing the evidence with PKC inhibitors that there is no tonic modulation of dopamine release through PKC. It may be that the stimulation frequency at 3 Hz is too low since it has been suggested for sympathetic neurones that PKC is endogenously activated only during high frequency stimulation (Musgrave & Majewski, 1989; Foucart *et al*., 1991). However in the present study, neither polymyxin B nor PKC down-regulation affected the S-I outflow of dopamine when the stimulation frequency was raised to 10 Hz instead of 3 Hz.

PKC may participate in D_2-receptor automodulation of dopamine release. When the dopamine autoreceptor was blocked by sulpiride this enhanced S-I release of dopamine and this effect was attenuated by polymyxin B and chelerythrine. Furthermore when PKC was down-regulated, sulpiride failed to enhance S-I dopamine release. This suggests that the enhanced S-I dopamine release in the presence of sulpiride involves the unmasking of a tonic PKC-mediated facilitation of dopamine release and implies that neuronally-released dopamine normally inhibits PKC activity through presynaptic D_2-receptors (see below for possible signalling pathways).

It is unlikely that the inhibition of sulpiride effects by PKC inhibitors is due to the elevated dopamine release after sulpiride, since when release was elicited to a similar level by increasing the frequency of stimulation (from 3 Hz to 10 Hz) no inhibitory effect of polymyxin B on S-I dopamine release was observed. We also altered the stimulation parameters to determine how robust the effect of PKC inhibitors was and when the slices were stimulated at 0.3 Hz for 400 s, the facilitatory effect of sulpiride was also attenuated by chelerythrine. It should be noted that all experiments were carried out in the presence of the dopamine uptake inhibitor nomifensine, which enhances automodulation (see Starke *et al*., 1989), and our attempt to examine the role of PKC in the absence of nomifensine was unsuccessful, as in this case the facilitatory effect of sulpiride was not present. It should be noted that many studies on dopamine release in rat striatum are conducted in the presence of dopamine uptake inhibitors (see Starke *et al*., 1989).

If dopamine auto-modulation occurs through inhibition of PKC then exogenous dopamine agonists should inhibit dopamine release in a PKC-dependent manner. Indeed, the inhibitory effect of the D_2-selective agonist quinpirole was partially attenuated in PKC down-regulated striatal slices. The lack of complete blockade suggests that part of its inhibitory effect involves a non-PKC mechanism since the actions of PDB were completely abolished under similar conditions. Another dopamine agonist apomorphine was unaffected by either polymyxin B or PKC down-regulation. It is possible that the lesser involvement of PKC in agonist (quinpirole, apomorphine)-mediated inhibition as compared with auto-inhibition (sulpiride) is because agonists access a different range of dopamine receptors to the endogenous transmitter. These agonist-activated receptors may utilize different signal transduction pathways. The differences between quinpirole and apomorphine inhibition of dopamine release in sensitivity to PKC down-regulation may be because quinpirole inhibits PKC activity in rat striatal synaptoneurosomes, whereas apomorphine has a more complex effect, inhibiting PKC at low concentrations (0.1 μM) and activating PKC at higher concentrations (10 μM) (Giambalvo & Wagner, 1994).

The above results suggest that D_2-receptors reduce a tonic PKC activity to cause inhibition of dopamine release. As stated above, D_2-receptor agonists inhibit PKC in striatal synaptoneurosomes (Giambalvo & Wagner, 1994). Although a direct effect on the enzyme cannot be ruled out, it is more likely that dopamine receptors decrease the formation of the endogenous PKC activator diacylglycerol which is formed by phospholipase C. In support of this hypothesis, activation of D_2-receptors inhibits inositol phosphate arm of the phosphlipase C pathway in striatal slices (Pizzi *et al*., 1987; von Euler & von Euler, 1991), although some workers find no effect (Kelly *et al*., 1988; Gupta & Mushra, 1990). Interestingly, electrically-evoked Ca^{2+} mobilization, which is presumably due to inositol phosphate, is inhibited by quinpirole in guinea-pig striatal slices (Fujiwara *et al*., 1987).

The question arises whether an action on PKC would agree with the current concepts concerning the signal transduction pathway which mediates D_2-autoreceptor effects. This pathway is unlikely to involve adenosine 3′:5′-cyclic monophosphate (cyclic AMP) (see Starke *et al*., 1989 for discussion) even though D_2-receptors are coupled to adenylate cyclase in many cell types (Jackson & Westlind-Danielsson, 1994). On the other hand, D_2-receptors enhance K^+ currents in many cells (Jackson & Westlind-Danielsson, 1994) and this could decrease transmitter release by hyperpolarizing the membrane, thus decreasing Ca^{2+} entry through voltage-dependent Ca^{2+} channels (see Starke *et al*., 1989). In striatal synaptosomes there is some evidence that K^+ channel modulation and not cyclic AMP is at least partially involved (Bowyer & Weiner, 1989) and microdialysis studies *in vivo* suggest that D_2-automodulation in rat striatum may involve ATP-sensitive K^+ channels (Tanaka *et al*., 1996). If K^+ channel modulation is the ultimate signal transduction step for D_2-autoreceptors, this may be consistent with the involvement of PKC which has been shown to inhibit K^+ channels in a variety of tissues (Shearman *et al*., 1989).

The work was supported by a grant from the National Health and Medical Research Council of Australia.

References

BOWYER, J.F. & WEINER, N. (1989). K^+ channel and adenylate cyclase involvement in regulation of Ca^{2+}-evoked release of [^{3}H]dopamine from synaptosomes. *J. Pharmacol. Exp. Ther*., **248,** 514 – 520.

BROUARD, A., PELAPRAT, D., VIAL, M., LHIAUBET, A.M. & ROSTENE, W. (1994). Effects of ion channel blockers and phorbol ester treatments on [^{3}H]dopamine release and neurotensin facilitation of [^{3}H]dopamine release from rat mesencephalic cells in primary culture. *J. Neurochem*., **62,** 1416 – 1425.

CHANDLER, L.J. & LESLIE, S.W. (1989). Protein kinase C activation enhances K^+-stimulated endogenous dopamine release from rat striatal synaptosomes in the absence of an increase in cytosolic Ca^{2+}. *J. Neurochem*., **52,** 1095 – 1912.

COPELAND, B.J., VOGELSBERG, V., NEFF, N.H. & HADJICONSTANTINOU, M. (1996). Protein kinase C activators decrease dopamine uptake into striatal synaptosomes. *J. Pharmacol. Exp. Ther*., **277,** 1527 – 1532.

CUBEDDU, L.X., HOFFMAN, I.S., TALMACIU, R.K., DILIBERTO, P. & LOVENBERG, T. (1991). Carbachol-phorbol ester interactions at muscarinic receptors modulating dopamine release from rabbit striatal slices. *Neurosci. Lett*., **131,** 245 – 248.

DI MARZO, V., VIAL, D., SOKOLOFF, P., SCHWARTZ, J.C. & PIOMELLI, D. (1993). Selection of alternative G-mediated signalling pathways at the dopamine D_2 receptor by protein kinase C. *J. Neurosci*., **13,** 4846 – 4853.

FOUCART, S., MUSGRAVE, I.F. & MAJEWSKI, H. (1991). Long term treatment with phorbol esters and the modulation of noradrenaline release. *Mole. Neuropharmacol.*, **1,** 95–101.

FUJIWARA, H., KATO, N., SHUNTOH, H. & TANAKA, C. (1987). D_2-dopamine receptor-mediated inhibition of intracellular Ca^{2+} mobilization and release of acetylcholine from guinea-pig neostriatal slices. *Br. J. Pharmacol.*, **91,** 287–297.

GIAMBALVO, C.T. & WAGNER, R.L. (1994). Activation of D_1 and D_2 dopamine receptors inhibits protein kinase C activity in striatal synaptoneurosomes. *J. Neurochem.*, **63,** 169–176.

GUPTA, S.K. & MUSHRA, R.K. (1990). The effect of dopamine D_1 and D_2 receptor agonists on inositol phosphate turnover in rat striatal slices. *Biochem. Int.*, **22,** 887–894.

HERBERT, J.M., AUGEREAU, J.M., GLEYE, J. & MAFFRAND, J.P. (1990). Chelerythrine is a potent and specific inhibitor of protein kinase C. *Biochem. Biophys. Res. Commun.*, **172,** 993–999.

HONG, D.F., HUAN, J., OU, B.R., YEH, J.Y., SAIDO, T.C., CHEEKE, P.R. & FORSBERG, N.E. (1995). Protein kinase C isoforms in muscle cells and their regulation by phorbol ester and calpain. *Biochem. Biophys. Acta*, **1267,** 45–54.

JACKSON, D.M. & WESTLIND-DANIELSSON, A. (1994). Dopamine receptors: molecular biology, biochemistry and behavioural aspects. *Pharmacol. Ther.*, **64,** 291–369.

KELLY, E., BATTY, I. & NAHORSKI, S.R. (1988). Dopamine receptor stimulation does not affect phosphoinositide hydrolysis in slices of rat striatum. *J. Neurochem.*, **51,** 918–924.

KOTSONIS, P. & MAJEWSKI, H. (1996). The structural requirements for phorbol esters to enhance noradrenaline and dopamine release from rat brain cortex. *Br. J. Pharmacol.*, **119,** 115–125.

LIU, Y.F., CIVELLI, O., GRANDY, D.K. & ALBERT, P.R. (1992). Differential sensitivity of the short and long human dopamine D_2 receptor subtypes to protein kinase C. *J. Neurochem.*, **59,** 2311–2317.

MUSGRAVE, I.F. & MAJEWSKI, H. (1989). Effect of phorbol esters and polymyxin B on modulation of noradrenaline release in mouse atria. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **339,** 48–53.

NICHOLS, R.A., HAYCOCK, J.W., WANG, J.K.T. & GREENGARD, P. (1987). Phorbol ester enhancement of neurotransmitter release from rat brain synaptosomes. *J. Neurochem.*, **48,** 615–621.

NISHIZUKA, Y. (1989). Studies and prospectives of the protein kinase C family for cellular regulation. *Cancer*, **63,** 1892–1903.

NISHIZUKA, Y. (1992). Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. *Science*, **258,** 607–614.

PIZZI, M., D'AGOSTIN, I.F., DA PRADA, M., SPANO, P.F. & HAEFELY, W.E. (1987). Dopamine D2 receptor stimulation decreases the inositol trisphosphate level of rat striatal slices. *Eur. J. Pharmacol.*, **136,** 263–264.

ROGUE, P., ZWILLER, J., MALVIYA, A.N. & VINCENDON, G. (1990). Phosphorylation by protein kinase C modulates agonist binding to striatal dopamine D_2 receptors. *Biochem. Int.*, **22,** 575–582.

SHEARMAN, M.S., SEKIGUCHI, K. & NISHIZUKA, Y. (1989). Modulation of ion channel activity: a key function of the protein kinase C enzyme family. *Pharmacol. Rev.*, **41,** 211–237.

STARKE, K., GÖTHERT, M. & KILBINGER, H. (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol. Rev.*, **69,** 864–989.

TALMACIU, R.K., HOFFMANN, I.S. & CUBEDDU, L.X. (1989). Differential effects of phorbol ester on prefrontal cortex and striatal dopamine terminals: dependence on rate and duration of stimulation. *J. Pharmacol. Exp. Ther.*, **251,** 1160–1165.

TANAKA, T., YOSHIDA, M., YOKOO, H., MIZOGUCHI, K. & TANAKA, M. (1996). ATP-sensitive K^+ channel openers block sulpiride-induced dopamine release in rat striatum. *Eur. J. Pharmacol.*, **297,** 35–41.

VERSTEEG, D.H.G. & ULENKATE, H.J.L.M. (1987). Basal and electrically stimulated release of [^{3}H]noradrenaline and [^{3}H]dopamine from amygdala slices in vitro: effects of 4β-phorbol 12, 13-dibutyrate, 4α-phorbol, 12,13-dideconate and polymyxin B. *Brain Res.*, **416,** 343–348.

VON EULER, G. & VON EULER, A. (1991). Dopamine D_2 receptors attenuate phosphatidylinositol 4,5-bisphosphate in synaptosomal membranes from rat neostriatum. *J. Neurochem.*, **56,** 136–140.

XU, M., YAMAMOTO, T. & KATO, T. (1990). In vivo striatal dopamine release by M_1 muscarinic receptors is induced by activation of protein kinase C. *J. Neurochem.*, **54,** 1917–1919.

ZURGIL, N., YAROM, M. & ZISAPEL, N. (1986). Concerted enhancement of calcium influx, neurotransmitter release and protein phosphorylation by a phorbol ester in cultured brain neurones. *Neuroscience*, **19,** 1255–1264.

(Received June 16, 1997
Revised September 1, 1997
Accepted September 11, 1997)