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Signal transduction interactions between CB_1 cannabinoid and dopamine receptors in the rat and monkey striatum

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Abstract

Signal transduction interactions between the CB_1 cannabinoid and D_1 and D_2 dopamine receptor systems were studied in rat (Sprague Dawley) and monkey (Macaca *fascilaris*) striatal membranes. The D_2 agonist quinelorane inhibited forskolin (10 μM)-stimulated adenylyl cyclase in a dose-dependent manner (26% and 20% maximal inhibition; EC_{50}=2 and 0.5 μM, in rats and monkeys, respectively) and maximal inhibition was completely blocked by the D_2 antagonist sulpiride (10 μM). The CB_1 agonist desacetyllevonantradol inhibited forskolin-stimulated adenylyl cyclase (18% and 36% maximal inhibition; EC_{50}=160 and 73 nM, in rats and monkeys, respectively) and the CB_1 antagonist SR141716A (10 μM) completely blocked the maximal inhibition. Combined addition of $>EC_{90}$ concentrations of quinelorane (10, 30 μM) and desacetyllevonantradol (1 μM) resulted in no greater inhibition than that produced by either drug alone, indicative of signal transduction convergence between the D_2 and CB_1 receptor systems. The D_1 agonist 6-Br-APB (3-allyl-6-bromo-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepin) produced a dose-dependent stimulation of adenylyl cyclase (45% and 26% stimulation; EC_{50}=24 and 32 nM, in rat and monkey, respectively), and maximal stimulation was completely blocked by the D_1 antagonist SCH23390 (1 μM). D_1 agonist-stimulated activity could be inhibited to basal levels with desacetyllevonantradol (1 μM), indicative of D_1 and CB_1 signal transduction convergence. The data suggest that CB_1 receptors are co-localized with D_1 or D_2 receptors on the same population of striatal membranes and can interact at the level of G-protein/adenylyl cyclase signal transduction. Similar results obtained with both rat and monkey membranes indicate that striatal dopamine and cannabinoid interactions are conserved for these two species.

Keywords: Adenylyl cyclase; G proteins; Basal ganglia; Striatum; Non-human primate; Rodents

1. Introduction

Both cannabinoid and dopamine receptors are found in large numbers in the striatum of rodents and primates (Herkenham et al., 1991; Levey et al., 1993; Ong and Mackie, 1999), where they are both hypothesized to regulate motor function (Alexander and Crutcher, 1990; Gough and Olley, 1977; Parent and Hazrati, 1995). In rats, stimulating the dopaminergic receptor systems increases motor activity (Woolverton and Kleven, 1988), whereas blocking normal dopamine function in the striatum by a D_1 dopamine antagonist leads to decreased motor function (Chipkin et al., 1988). Cannabinoid agonists microinjected into the striatum produce immobility (Gough and Olley, 1978). Given that these two receptor systems appear to have opposing roles in regulating motor function in the rat, many groups have investigated the interactions between cannabinoid and dopamine receptor systems in vivo (Anderson et al., 1995, 1996; Maneuf et al., 1997; Navarro et al., 1993; Rodriguez de Fonseca et al., 1994; Sakurai et al., 1985; Sanudo-Pena et al., 1998). Co-administration studies demonstrate an opposition between D_1 or D_2 dopamine receptor agonists and cannabinoid agonists in immobility and catalepsy in rodents (Anderson et al., 1996). Primates exhibit freezing and catalepsy when administered dopamine antagonists such as haloperidol (Casey, 1992). In contrast to the behavioral effects elicited by canna-

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binoid agonists in rodents, primates administered cannabinoid agonists do not exhibit freezing or catalepsy. Rather, primates exhibit bradykinesia, sedation, and a decrease in general and locomotor activity (Meschler et al. 2000a, 2001). Although the effects of CB_1 agonists in rodents can be reversed by D_1 or D_2 dopamine receptor agonists (Meschler et al., 2000b), D_2 agonists potentiate the effects of a cannabinoid agonist in primates (Meschler et al. 2000a, 2001).

In order to explain the species-specific behavioral differences between cannabinoid and dopamine interactions, we hypothesized that cellular signal transduction interactions in the striatum may differ between rodents and primates. D_1 agonists are considered to stimulate adenylyl cyclase via G_s (Strange, 1993), and D_2 and CB_1 receptors are considered to inhibit adenylyl cyclase via G_i (Pertwee, 1999; Strange, 1993). However, whether these receptors modulate cyclic AMP production in a coordinate manner within the same neurons within the striatum is unknown. Immunohistochemical staining of CB_1 receptors in the striatum demonstrates differences in the regional distribution of the CB_1 cannabinoid receptor in the striatum in monkeys (Ong and Mackie, 1999) compared with rats (Pettit et al., 1998). The CB_1 receptor distribution is denser in the lateral versus the medial striatum in the rat (Pettit et al., 1998), whereas the CB_1 receptors are distributed more diffusely throughout the striatum in the monkey (Ong and Mackie, 1999). Thus, the co-localization of both dopamine and cannabinoid receptors on the same neurons may differ between species.

In this report, we have investigated the ability of CB_1 cannabinoid receptors to co-regulate adenylyl cyclase activity with either the D_2 or D_1 type dopamine receptors in rat and monkey striatal membranes. We determined that the inhibition of cyclic AMP formation by D_2 and CB_1 agonists was not additive for either rat or monkey striatal membranes, suggesting co-localization of these two receptors with adenylyl cyclase in a majority of membrane vesicles. A cannabinoid agonist inhibited D_1-stimulated cyclic AMP formation nearly completely, suggesting that the majority of membrane vesicles in which adenylyl cyclase is stimulated by the D_1 receptors are inhibited by CB_1 receptors. These data demonstrate similar results in both rat and primate striatum, suggesting that the different behavioral responses between cannabinoid and dopamine receptor systems in rat versus monkey cannot be explained by species differences in signal transduction interactions in the striatum.

2. Methods

2.1. Dissection of striatal tissue

Male Sprague Dawley rats from Harlan (Indianapolis, IN) were maintained in accordance with the NIH Guide for Care and Use of Laboratory Animals. Rats were anesthetized with ketamine and phenobarbital and then decapitated. The brains were quickly removed and the striatum was dissected free hand on ice and placed in ice-cold TM buffer (50 mM Tris Cl, pH 7.4 and 3 mM $MgCl_2$).

A male cynomolgus monkey (Macaca *fasciluaris*) was anesthetized with ketamine and phenobarbital and the brain was quickly removed. Coronal brain slices (approximately 5 mm thick) were made at the level of the striatum; the striatum was dissected free hand from the brain slices on ice and placed in TM buffer on ice.

2.2. Membrane preparation and adenylyl cyclase determinations

Rat and monkey membranes were prepared using identical methods. The striatal tissues were gently homogenized with a dounce homogenizer in cold TM buffer on ice. Homogenized brain tissue was centrifuged at 48,000*g* at 2°C for 10 min. The membrane pellet was resuspended in low pH-buffer (50 mM sodium acetate, pH 4.5, 5 mM $MgCl_2$, 1 mM dithiothreital, and 1 mM EGTA) and incubated on ice for 10 min according to the method of Childers and colleagues in an effort to maximize detectability of the response to G_i coupled receptors (Selley et al., 1993). The membranes were subsequently diluted with a five-fold excess of TM buffer and sedimented at 48,000*g* at 2°C for 10 min. The pellet was resuspended in TM buffer and aliquots of the membranes were stored at −80°C. Membranes were thawed just prior to addition to the adenylyl cyclase assay.

Adenylyl cyclase activity was determined by conversion of α-[^{32}P]ATP to [^{32}P]cyclic AMP as previously described (Howlett, 1985) except that striatal membranes (0.15 mg protein/ml) were used. RO20-1724 (0.1 mM) plus isobutylmethylxanthine (0.1 mM) were used as phosphodiesterase inhibitors, and 10 μM forskolin was present where indicated as an adenylyl cyclase activator. Activity was determined as pmol cyclic AMP formed per min per mg protein, and then reported as either a percentage of forskolin-stimulated activity or a percentage of basal activity.

2.3. Materials

Desacetyllevonantradol (DALN) was a generous gift from Pfizer (Groton, CT) and quinelorane was a gift from Eli Lilly (Indianapolis, IN). 3-Allyl-6-bromo-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine (6-Br-APB), sulpiride, SCH23390, RO20-1724 and isobutylmethylxanthine were purchased from RBI (Natick, MA).

2.4. Graphing and statistical analysis

Curves were generated by least squares non-linear regression analysis of a sigmoidal curve, interacting on the four parameters of maximum, minimum, slope factor, and EC_{50} (GraphPad Prism software). Multiple comparisons were made by ANOVA, and the Dunnett or the Bonferroni multiple comparison post hoc tests were used as indicated in the figure legends (GraphPad Prism software). The level of significant difference from vehicle (Dunnett's test) or between variables (Bonferroni test) is indicated in the figure legends. Graphs show data normalized such that vehicle is 100%

3. Results

3.1. CB_1 cannabinoid receptor–D_2 dopamine receptor signal transduction interactions

In order to determine the potency and effectiveness of the D_2 dopamine receptor agonist quinelorane to inhibit adenylyl cyclase activity in striatal membranes, a log dose–response curve was generated employing either rat (Fig. 1A) or monkey (Fig. 1B) membranes. Forskolin-stimulated adenylyl cyclase activity was inhibited in a dose-dependant manner with an EC_{50} of 2 μM for rat and 0.5 μM for monkey. Maximal inhibition of forskolin-stimulated adenylyl cyclase activity was 27% for rat and 20% for monkey. These differences in EC_{50} and maximal inhibition produced by quinelorane between rat and monkey did not reach statistical significance. In order to confirm that quinelorane-induced inhibition of forskolin-stimulated activity was due to stimulation of D_2 dopamine receptors, the response was blocked with the D_2-specific antagonist sulpiride. Sulpiride completely antagonized the response to concentrations of quinelorane at or above EC_{90} concentrations (10 and 30 μM) in both rat (Fig. 2A) and monkey (Fig. 2B) striatal membranes. Thus, the inhibition of striatal adenylyl cyclase by quinelorane is due to activation of D_2 dopamine receptors. The lack of effect of sulpiride alone demonstrated that stimulation of D_2 receptors by endogenous dopamine was not occurring in these membranes.

We next wanted to determine the potency and effectiveness of the CB_1 cannabinoid agonist DALN to inhibit forskolin-stimulated adenylyl cyclase in striatal membranes. Log dose–response curves were generated over a range of doses of DALN in both the rat (Fig. 3A) and monkey (Fig. 3B) membranes. The EC_{50} for this inhibition was 160 nM for rat and 73 nM for monkey with a maximal inhibition of 18% (rat) and 36% (monkey) of forskolin-stimulated adenylyl cyclase activity. Neither the EC_{50} nor the maximum inhibition were significantly different between rat and monkey. The CB_1 specific antagonist, SR141716A, effectively antagonized a dose of DALN above the EC_{90} concentration (1 μM) in both rat (Fig. 4A) and monkey (Fig. 4B) membranes, but had no effect when administered alone. This confirms that the inhibition of adenylyl cyclase by DALN is due to activation of CB_1 cannabinoid receptors, and also precludes the possibility of stimulation of CB_1 receptors by endogenous cannabinoid agonists.

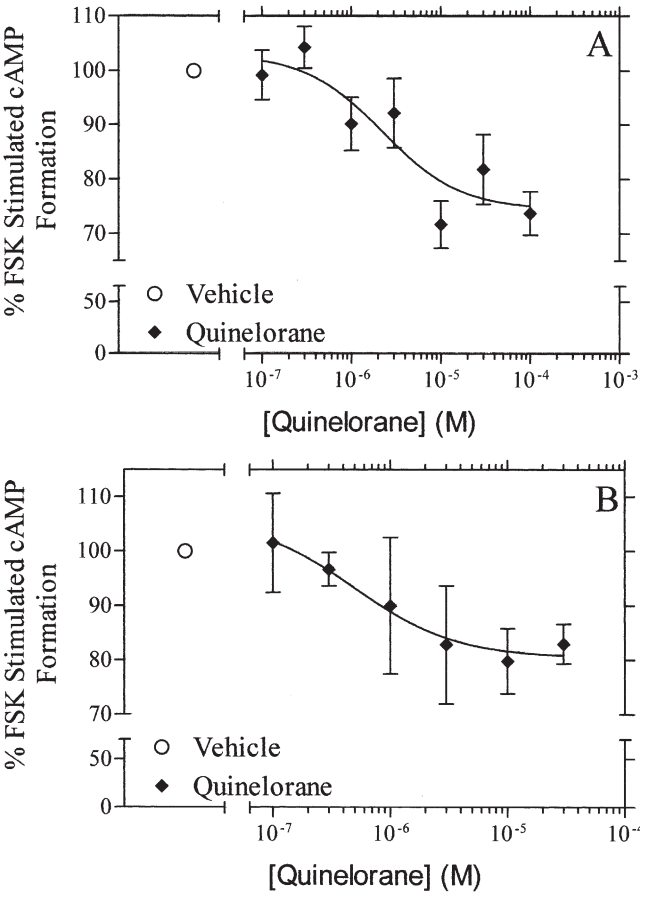

Fig. 1. Dose-dependent inhibition of forskolin (10 μM) stimulated adenylyl cyclase activity by the D_2 dopamine receptor agonist quinelorane in rat (A) and in monkey (B) striatal membranes. (A) The curve was generated from data averaged from four individual experiments, and the EC_{50} was 2.5 μM (CI: 0.4–16 μM); maximal inhibition was 27% (CI: 16–39%). (B) The curve was generated from data averaged from four individual experiments, and the EC_{50} was 0.5 μM (CI: 9.4 nM–27 μM); maximal inhibition was 20% (CI: 10–29%).

If D_2 dopamine and CB_1 cannabinoid receptors were present on two different populations of neurons in the striatum, then the simultaneous stimulation of both receptors would be additive in the striatal membrane preparation. Alternatively, if both receptor types were present together on neurons such that they regulated the same population of adenylyl cyclase molecules, then it would be predicted that maximal inhibition would be limited by the number of affected adenylyl cyclase molecules and no additivity of maximal receptor stimulation would be observed. Mixed populations of neurons which possess either or both receptor types would result in a

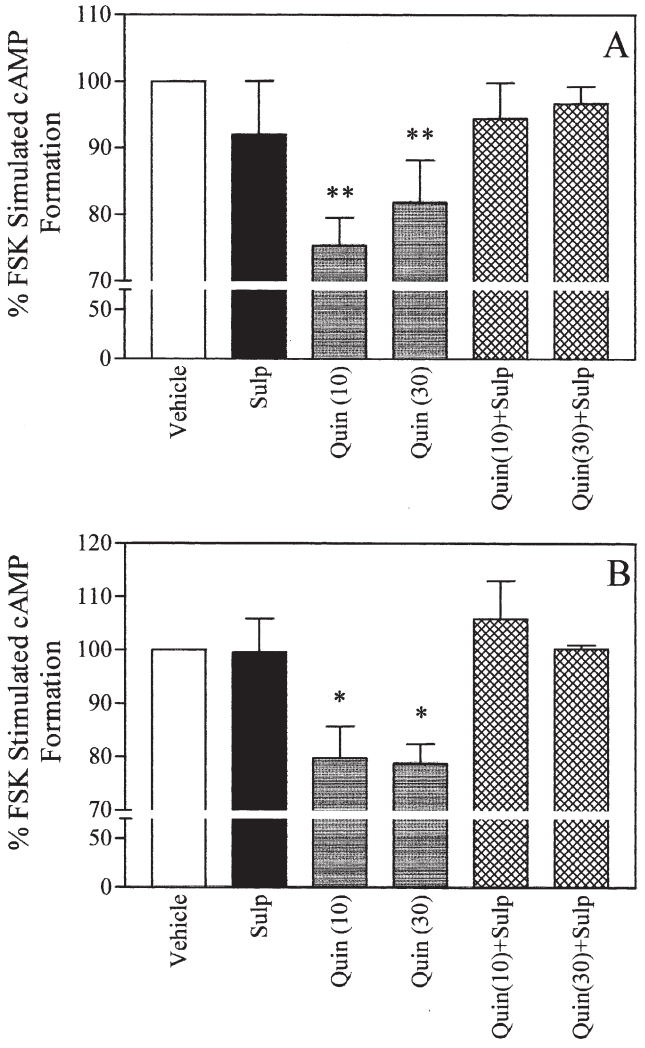

Fig. 2. Antagonism of quinelorane-induced inhibition of forskolin (10 μM) stimulated adenylyl cyclase activity by the D_2 dopamine receptor antagonist sulpiride in rat (A) and in monkey (B) striatal membranes. The concentrations of drugs were as follows: quinelorane (Quin) 10 or 30 μM, as indicated in parentheses, and 10 μM sulpiride (Sulp). Statistically significant differences from vehicle control were determined employing the Dunnett post hoc test and are denoted as: * $p<0.05$; ** $p<0.01$. The bar graph represents the mean±SEM from four individual experiments.

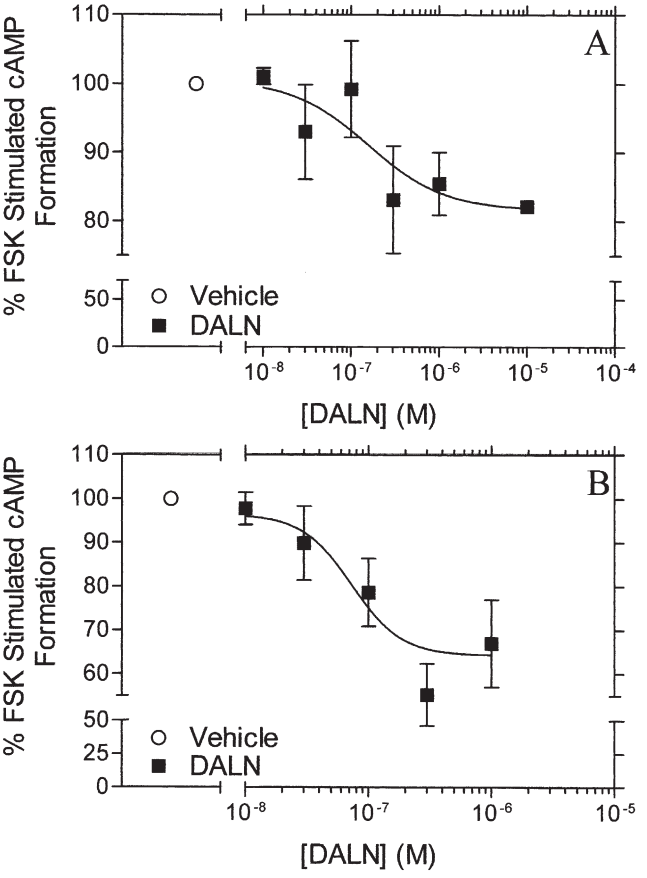

Fig. 3. Dose-dependent inhibition of forskolin (10 μM) stimulated adenylyl cyclase activity by the CB_1 cannabinoid receptor agonist DALN in rat (A) and in monkey (B) striatal membranes. (A) The curve was generated from data averaged from four individual experiments, and the EC_{50} was 160 nM (CI: 3.2 nM–7.7 μM); maximal inhibition was 18% (CI: 4–33%). (B) The curve was generated from data averaged from four individual experiments, and the EC_{50} was 73 nM (CI: 8.8–610 nM); maximal inhibition was 36% (CI: 20–52%).

less than quantitative additivity. We used two concentrations of quinelorane (10 and 30 μM), both of which were at or above the EC_{90} concentration in monkey and rodent, and could be effectively blocked by sulpiride. The concentration chosen for DALN (1 μM) was above the EC_{90} for rat and monkey and could be effectively blocked by the CB_1 cannabinoid receptor antagonist, SR141716A. Combining D_2 and CB_1 agonists at their maximally effective concentrations failed to produce an additive inhibition in either rat (Fig. 5A) or in monkey (Fig. 5B) membranes. The drug concentrations selected for the additivity studies ensured that a maximal response for that drug could be generated. It is observable from the curves in Figs. 1 and 3 that inhibition was only a fraction of the vehicle response (18–36%). This would mean that further inhibition was indeed possible. It could be predicted that a completely additive response for CB_1 and D_2 combinations would have increased the inhibition to 45% for rats and 56% for monkeys. These values would have been greater than the confidence interval surrounding the maximum for either of the two drugs (Figs. 1 and 3). An ANOVA was used to determine if significant differences could be observed between individual drugs at their maximally effective concentration and the combination of drugs. The means in those comparisons are not significantly lower than either drug alone, and do not even suggest a trend for additive inhibition.

Neither the CB_1 cannabinoid receptor antagonist, SR141716A, nor the D_2 dopamine receptor antagonist, sulpiride, were able to antagonize the inhibition produced by the quinelorane plus DALN combination in either the rat (Fig. 5A) or the monkey (Fig. 5B) membranes. Thus, antagonism of the effects of either DALN

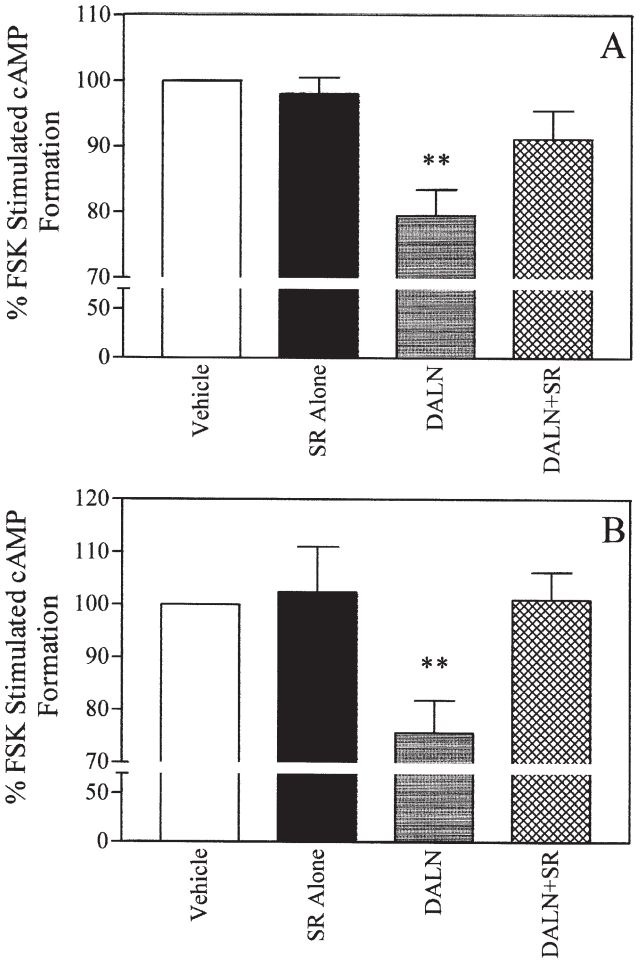

Fig. 4. Antagonism of DALN-induced inhibition of forskolin (10 μM) stimulated adenylyl cyclase activity by the CB_1 cannabinoid receptor antagonist SR141716A in rat (A) and in monkey (B) striatal membranes. The concentrations of drugs were as follows: DALN 1 μM and SR141716A (SR) 10 μM. Statistically significant differences from vehicle control were determined employing the Dunnett post hoc test and are denoted as: *$p<0.05$; **$p<0.01$. The bar graph represents data averaged from four individual experiments.

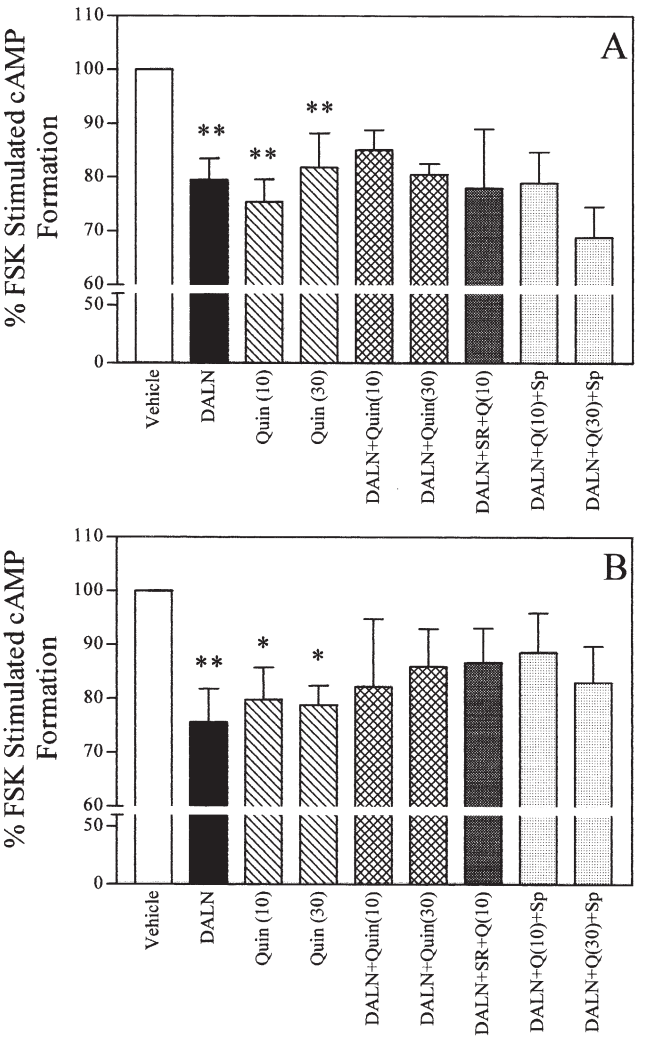

Fig. 5. Effect of the combination of the CB_1 cannabinoid receptor agonist DALN and the D_2 dopamine receptor agonist quinelorane to inhibit forskolin (10 μM) stimulated adenylyl cyclase activity in rat (A) and in monkey (B) striatal membranes. The concentrations of drugs were as follows: DALN 1 μM; quinelorane (Quin) 10 or 30 μM, as indicated in parentheses; SR141716A (SR) 10 μM; and sulpiride (Sp) 10 μM. Statistically significant differences from vehicle control were determined employing the Dunnett post hoc test and are denoted as: *$p<0.05$; **$p<0.01$. None of the experimental samples differed significantly from DALN ($p>0.05$). The bar graph represents data averaged from four individual experiments.

or quinelorane allowed the full expression of inhibition by the unantagonized receptor agonist.

We wished to determine if CB_1 cannabinoid and D_2 dopamine receptors could activate identical pools of G proteins. In these analyses, DALN and quinelorane produced markedly different patterns of stimulation of [^{35}S]GTPγS binding. DALN produced a 40–50% increase in [^{35}S]GTPγS binding (Meschler et al., 2000c), whereas quinelorane (100 nM–100 μM) failed to produce a response that was significantly above basal activity (data not shown).

3.2. CB_1 cannabinoid receptor–D_1 dopamine receptor signal transduction interactions

In order to determine the potency and effectiveness of the D_1 agonist 6-Br-APB in rat and monkey striatal membranes, a log dose–response curve was generated (Fig. 6). 6-Br-APB produced a dose-dependant stimulation of adenylyl cyclase in striatal membranes, with EC_{50} values of 24 nM for rat and 32 nM for monkey, and a maximum stimulation of 45% for rat and 26% for monkey above non-stimulated basal activities. The D_1 antagonist SCH23390 (1 μM) reversed the effects of a concentration of 6-Br-APB that was approximately 10 times the EC_{90}, confirming that the effects of 6-Br-APB are mediated through the D_1 receptor. SCH23390 (1 μM) had no effect on non-stimulated basal adenylyl cyclase activity, demonstrating that no detectable endogenous

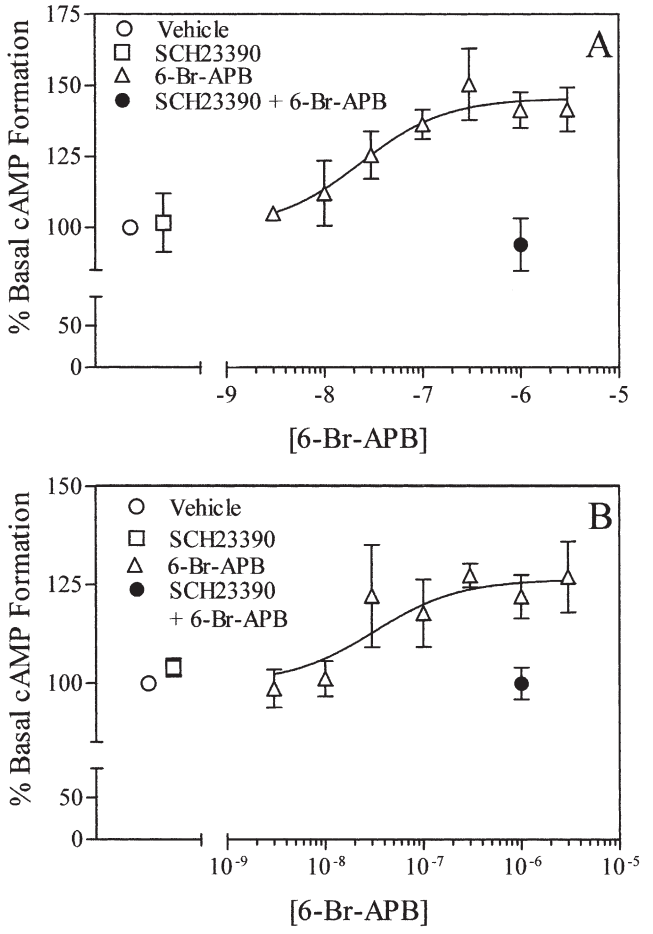

Fig. 6. Dose-dependent stimulation of adenylyl cyclase activity by the D_1 dopamine receptor agonist 6-Br-APB in rat (A) and in monkey (B) striatal membranes. The D_1 dopamine receptor antagonist SCH23390 (1 μM) antagonized the effects of 6-Br-APB (1 μM) while having no effect when administered alone. (A) The curve was generated from data averaged from four individual experiments, and the EC_{50} was 24 nM (CI: 7.9–710 nM); maximal stimulation above basal was 45% (CI: 36–55%). (B) The curve was generated from data averaged from four individual experiments, and the EC_{50} was 32 nM (CI: 5.0–200 nM); maximal stimulation above basal was 26% (CI: 17–36%).

dopamine stimulated D_1 receptor activity exists in these washed membrane preparations.

Reports from Childer's laboratory have suggested that G_i activity may be enhanced by acid treatment and G_s activity may be hindered in membranes treated in this manner (Childers, 1988; Rasenick and Childers, 1989). We compared acid treated versus non-treated rat striatal membranes and found little difference in D_1 agonist stimulated adenylyl cyclase activity (data not shown). In Childer's studies (Childers, 1988; Selley et al., 1993), the concentrations of GTP added to the assay were low relative to the protein content (50 μM GTP and 2 mg/ml protein) and the NaCl concentration was high (120 mM) compared to our assay conditions (100 μM GTP and 0.15 mg/ml protein; <50 mM NaCl). Because we were able to obtain sufficient and detectable stimulation of adenylyl cyclase activity in acid treated membranes, we show the results from D_1 receptor stimulation in acid treated membranes for a more appropriate comparison with the other experiments in this series.

Studies were performed to determine if CB_1 receptors could inhibit the D_1 receptor-stimulated adenylyl cyclase activity in striatal membranes, indicative of co-localization of these two receptor types. Two concentrations of 6-Br-APB were used (0.1 and 1.0 μM), both of which produced a significant increase above non-stimulated adenylyl cyclase activity. DALN significantly inhibited 6-Br-APB-stimulated activity to basal levels in the rat (Fig. 7A) and monkey (Fig. 7B), although DALN alone produced no significant effect on basal activity. The CB_1 antagonist SR141716A (10 μM) significantly blocked the DALN-induced inhibition of 6-Br-APB-stimulated adenylyl cyclase activity in the rat (Fig. 7A) and in the monkey (Fig. 7B). These data indicate that DALN-induced inhibition of 6-Br-APB-stimulated adenylyl cyclase activity is mediated by receptors that are co-localized on neurons in the striatum of both species.

4. Discussion

These data demonstrate that there are no significant additive effects between CB_1 and D_2 receptor systems with respect to their ability to inhibit adenylyl cyclase activity in rat and monkey striatum. Because EC_{90} concentrations of the CB_1 agonist and the D_2 agonist each produced an approximate 20% decrement in activity, we would expect to see a 40% decrement when the drugs were administered together if the effects of these drugs were additive. However, when quinelorane and DALN were co-administered, the decrease in activity was not significantly different from either drug administered alone, despite the fact that there was still significant adenylyl cyclase activity that could be potentially inhibited. The lack of additivity suggests that the D_2 and CB_1 receptor signal transduction pathways converge onto the same pool of adenylyl cyclase, implying that there must be a population of CB_1 and D_2 receptors located on the same vesicles in the membrane preparations used for the assay. Because these membranes were homogenized by a gentle dounce technique, each vesicle formed in the membrane preparation contains a representative sampling of the membrane bound proteins from a discrete region of a single neuron, such as the axon terminal membrane versus the dendritic membrane versus the nuclear membrane (Calderon and DeVries, 1997). Furthermore, vesicular fusion is unlikely to occur with the gentle dounce technique (Cioffi and Wolfersberger, 1983; DeBacker and Wolosin, 1991). Hence these data suggest that CB_1 and D_2 receptors are co-localized on the same neurons and the same regions of

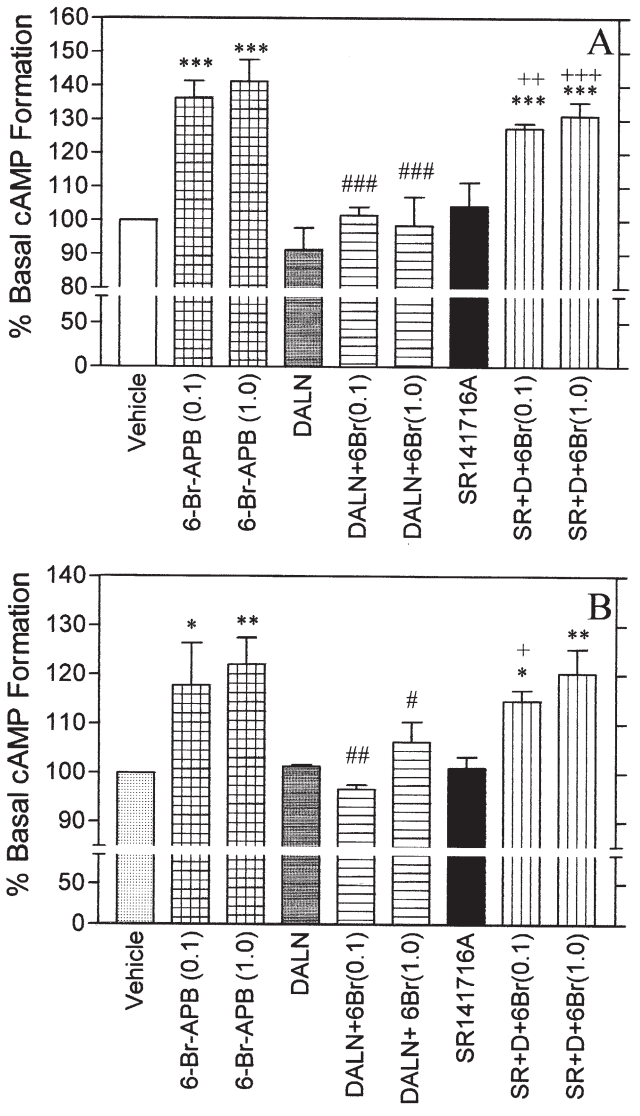

Fig. 7. Inhibition of 6-Br-APB-stimulated adenylyl cyclase activity by the CB_1 cannabinoid receptor agonist DALN and its antagonism by SR141716A in rat (A) and in monkey (B) striatal membranes. The concentrations of drugs were as follows: DALN (D) 1 μM, 6-Br-APB (6-Br) 0.1 or 1.0 μM, as indicated in parentheses, and SR141716A (SR) 10 μM. Statistically significant differences were determined employing the Bonferroni multiple comparisons test. Statistically significant difference from vehicle is denoted as: *$p<0.05$; **$p<0.01$; ***$p<0.001$; significant difference from 6-Br-APB is denoted as: #$p<0.05$; ##$p<0.01$; ###$p<0.001$; and significant difference from DALN plus 6-Br-APB is denoted as: +$p<0.05$; ++$p<0.01$; +++$p<0.001$.

neurons in the striatum and that signal transduction convergence occurs in these regions.

It is not entirely clear where in the signal transduction pathway, convergence between the CB_1 and D_2 receptor systems may be occurring. In rat cerebellar membranes, co-incubation of both μ-opioid agonists and CB_1 agonists produced an additive response in the [^{35}S]GTPγS binding assay (indicative of G-protein activation) but a complete lack of additivity in the adenylyl cyclase assay (Childers et al., 1992). This scenario also appears to be the case with signal transduction convergence between CB_1 and δ-opioid receptor signal transduction in N18TG2 cells, in which CB_1 receptors activate a larger pool of G proteins than the δ-opioid receptors and the response to maximally activating both receptors is completely additive (Shapira et al., 1998), whereas the inhibition of adenylyl cyclase is not additive (Devane et al., 1986). One possible interpretation is that CB_1 and the opioid (Childers et al., 1992; Shapira et al., 1998; Devane et al., 1986) or D_2 (data reported here) receptor signal transduction cascades converge at sharing the same pool of adenylyl cyclase. A second possibility is that these two receptor systems activate the same pool of G_i proteins that are involved in regulation of adenylyl cyclase. We do not believe that CB_1 and D_2 receptors activate identical pools of G proteins because under experimental conditions in which DALN produced a marked increase in [^{35}S]GTPγS binding, quinelorane failed to produce a response that was significantly above basal activity. Thus, the pool of G_i that is being activated by D_2 receptors is either small in quantity or slow in exchanging guanine nucleotides, making it undetectable in the [^{35}S]GTPγS binding assay. In contrast, the pool of G_i proteins activated by CB_1 receptors is sufficiently large and rapidly exchanging making it easily detectable in this assay. However, it is possible that the pool of G proteins that ultimately regulates adenylyl cyclase is common to both CB_1 and D_2 receptors. In rat brain (Mukhopadhyay et al., 2001) and in N18TG2 cells (Mukhopadhyay and Howlett, 2001), the CB_1 receptor associates with three different subtypes of $G_{\alpha i}$ isoforms ($G_{\alpha i1}$, $G_{\alpha i2}$ and $G_{\alpha i3}$). A specific region of the CB_1 receptor (the juxtamembrane C-terminal) activates $G_{\alpha i3}$, whereas the third loop of the CB_1 receptor associates with $G_{\alpha i1}$ and $G_{\alpha i2}$ (Mukhopadhyay and Howlett, 2001). Hence, it is possible that both D_2 and CB_1 receptors activate a specific subtype of G_i protein by virtue of a similar activation domain, and that this pool of G proteins is responsible for the majority of receptor mediated inhibition of adenylyl cyclase activity in the striatum.

The data reported here appear contradictory to what has been previously reported for cAMP accumulation in primary cultures of fetal rat striatum. Glass and Felder (1997) reported that a D_2 agonist plus a cannabinoid agonist produced a paradoxical increase in cAMP accumulation. Furthermore, they demonstrated that a CB_1 agonist increased cAMP accumulation after pertussis toxin treatment (which inactivates $G_{\alpha i}$ proteins) (Glass and Felder, 1997). We attempted to replicate this using rat striatal membranes, but found that DALN and quinelorane both failed to produce any effects (either stimulatory or inhibitory) after pertussis toxin-A treating the membranes (data not shown; see Mukhopadhyay and Howlett, 2001, for methods). The disparity in results might be explainable if cultured cells possessed a differ-

ent composition of G proteins or different adenylyl cyclase isoforms compared with the membranes isolated from intact adult striata.

The inhibitory actions of DALN on D_1 agonist stimulated (6-Br-APB) adenylyl cyclase activity suggests that D_1 and CB_1 receptor signal transduction pathways converge onto the same pool of adenylyl cyclase. The fact that 6-Br-APB stimulated activity is inhibited to basal activity by DALN suggests that a majority of the pool of adenylyl cyclase affected by these two receptor systems is shared between the CB_1 and D_1 receptor system signal transduction cascades.

Most important to our observations of species differences in motor behaviors (Meschler et al. 2000a,b, 2001) is the similarity in the signal transduction between rat and monkey striatum. In both monkey and rat striatum, we demonstrated nearly equivalent EC_{50} values for DALN, quinelorane and 6-Br-APB, suggesting that these drugs have the same potencies in these two species. We saw the same lack of additivity between D_2 and CB_1 receptor systems, suggesting that these receptor systems are co-localized on the same neurons and have convergent signal transduction cascades in both the rat and primate. Furthermore, the ability of DALN to inhibit 6-Br-APB stimulated adenylyl cyclase activity equally in both rat and monkey suggests that co-localization and receptor signal transduction convergence exist between these two receptor systems in both species. These findings lead to the conclusion that rodent and monkey D_2 and CB_1 receptor mediated changes in motor behaviors may be more complex than can be discerned from studies of striatal signal transduction. These receptor systems may mediate their influence on motor behaviors by differential effects involving multiple regions of the basal ganglia, such as the globus pallidus, substantia nigra or subthalamic nucleus.

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