Cocaine modulates allosteric D_2-σ_1 receptor-receptor interactions on dopamine and glutamate nerve terminals from rat striatum

Sarah Beggiato[a,1], Andrea Celeste Borelli[a,1], Dasiel Borroto-Escuela[b], Ilaria Corbucci[a], Maria Cristina Tomasini[a], Matteo Marti[a], Tiziana Antonelli[c], Sergio Tanganelli[c], Kjell Fuxe[b], Luca Ferraro[a]

[a]Department of Life Sciences and Biotechnology (SVEB), University of Ferrara, Ferrara, Italy; [b]Neuroscience, Karolinska Institutet, Stockholm, Sweden; [c]Department of Medical Sciences, University of Ferrara, Ferrara, Italy;

[1]These authors equally contributed to the study.

Abbreviations: cAMP Response Element-Binding protein (CREB); central nervous system (CNS); D2 receptor (D2R); dopamine (DA); dopamine transporter (DAT); Human Embryonic Kidney cells 293 (HEK293T); net extra-outflow (NER); σ1 receptors (σ1Rs); voltage dependent calcium 1.3 (Cav 1.3).

Address correspondence and reprint requests to: Dr Luca Ferraro, Department of Life Sciences and Biotechnology (SVEB), University of Ferrara, Via Fossato di Mortara 17-19, Ferrara, Italy.
Tel: 0039 0532 455276; E-mail: frl@unife.it

Abstract

The effects of nanomolar cocaine concentrations, possibly not blocking the dopamine transporter activity, on striatal D_2-σ_1 heteroreceptor complexes and their inhibitory signalling over Gi/o, have been tested in rat striatal synaptosomes and HEK293T cells. Furthermore, the possible role of σ_1 receptors (σ_1Rs) in the cocaine-provoked amplification of D_2 receptor (D_2R)-induced reduction of K^+-evoked [^{3}H]-DA and glutamate release from rat striatal synaptosomes, has also been investigated.

The dopamine D_2-likeR agonist quinpirole (10 nM-1μM), concentration-dependently reduced K^+-evoked [^{3}H]-DA and glutamate release from rat striatal synaptosomes. The σ_1R antagonist BD1063 (100 nM), amplified the effects of quinpirole (10 and 100 nM) on K^+-evoked [^{3}H]-DA, but not glutamate, release.

Nanomolar cocaine concentrations significantly enhanced the quinpirole (100 nM)-induced decrease of K^+-evoked [^{3}H]-DA and glutamate release from rat striatal synaptosomes. In the presence of BD1063 (10 nM), cocaine failed to amplify the quinpirole (100 nM)-induced effects.

In cotransfected σ_1R and D_{2L}R HEK293T cells, quinpirole had a reduced potency to inhibit the CREB signal versus D_{2L}R singly transfected cells. In the presence of cocaine (100 nM), the potency of quinpirole to inhibit the CREB signal was restored. In D_{2L} singly transfected cells cocaine (100 nM and 10 μM) exerted no modulatory effects on the inhibitory potency of quinpirole to bring down the CREB signal.

These results led us to hypothesize the existence of functional D_2-σ_1R complexes on the rat striatal DA and glutamate nerve terminals and functional D_2-σ_1R-DA transporter complexes on the striatal DA terminals. Nanomolar cocaine concentrations appear to alter the allosteric receptor-receptor interactions in such complexes leading to enhancement of Gi/o mediated D_2R signalling.

Keywords: Receptor heteromers; Synaptosomes; Release; Quinpirole; BD1063; HEK293T cells.

1. Introduction

Cocaine-induced increases in striatal and nucleus accumbens dopamine (DA) levels are thought to underlie the rewarding/reinforcing actions of the drug in humans, leading to its abuse and addiction [1-4]. Previous studies suggested the presynaptic DA transporter (DAT) as the principal target for cocaine effects [5-7]. However, over the last years it has become evident that cocaine, in the nanomolar range, can enhance DA D_2 receptor (D_2R) functions as demonstrated by biochemical and behavioural experiments. In particular, cocaine modulates D_2R agonist recognition, G-protein coupling, and signalling in cell lines lacking the DAT [8-10]. Furthermore, *in vitro* and *in vivo* studies indicate that cocaine enhances the D_2-likeR agonist quinpirole-induced reduction of striatal [^{3}H]-DA release and nucleus accumbens glutamate release, respectively [10,11]. Finally, cocaine, but not the DAT blocker GBR 12783, enhanced quinpirole (1 mg/kg)-induced hyperlocomotion in the rat. Such actions are likely to be relevant for the rewarding and relapsing effects of cocaine in view of the major role of nucleus accumbens and dorsal striatal D_2Rs in mediating these mechanisms [12-14].

Sigma-1 (σ_1) receptors (σ_1Rs) are highly expressed in the central nervous system (CNS), including the dorsal striatum and the nucleus accumbens [15,16]. It has been proposed that σ_1R activation is involved in different aspects of cocaine abuse [17,18], and σ_1R antagonists were found to reduce the behavioural effects of cocaine [18,19]. Among other mechanisms (Matsumoto et al., 2014), it seems likely that a D_2R-σ_1R heteromerization might be involved in these effects. In fact, it has been proven that D_2Rs can physically interact with σ_1Rs in mouse striatum and that cocaine by interacting with this heteromer modulates downstream signalling in both cultured cells and in mouse striatum [16]. Although the authors proposed that these heteroreceptor complexes are localized at the post-synaptic level, their possible existence also at the pre-synaptic levels cannot be excluded. The allosteric action of cocaine at D_2Rs might thus be indirect and mediated by the σ_1R protomer at the D_2R-σ_1R heteroreceptor complex. In line with this view, it was reported that in D_2Rs/σ_1R cotransfected cells cocaine, at 30 μM concentration, partially counteracted the decrease in the Gi activation induced by quinpirole, and this effect was mediated by the σ_1 protomer at the D_2R-σ_1R heteroreceptor complex [16]. This result is in sharp contrast with the enhancement of quinpirole-induced reduction of K^+-evoked [^{3}H]-DA release from rat striatal synaptosomes and of quinpirole-induced inhibition of nucleus accumbens dialysate glutamate levels observed following treatments with cocaine in a low (*i.e.* nanomolar) concentration range [10-11].

In the current study we tested the effects of nanomolar cocaine concentrations, possibly not blocking the DAT activity [11], on striatal D_2R-σ_1R heteroreceptor complexes and their inhibitory

signalling over Gi/o. Furthermore, the possible role of σ_1Rs in the cocaine provoked amplification of quinpirole-induced reduction of K^+-evoked [^{3}H]-DA and glutamate release from rat striatal synaptosomes, has also been investigated.

2. Materials and methods

2.1. Synaptosome experiments

2.1.1. Animals

Male Sprague–Dawley rats (250-300 g; Harlan, San Pietro al Natisone, Udine, Italy) were used. The animals had free access to food and water and were housed in a room with a 12:12 h light-dark cycle (lights on at 08.00 a.m.) and at a temperature of 22±1C° for at least 1 week before experiments. All animal experiments complied with the ARRIVE guidelines and were performed in accordance with the EU directive of September 22, 2010 (2010/63/EU) on the protection of animals used for scientific purposes, after approval of the experimental protocols by the Italian Ministry of Health. Efforts were made to minimize the number of animals used and to reduce pain and discomfort.

2.1.2. Striatal synaptosome preparation

On the day of the experiment, the animal was sacrificed and the striata were rapidly dissected out from the brain. Thereafter, a crude synaptosomal (P2) fraction was prepared as follows: the tissue was suspended in ice-cold buffered sucrose solution (0.32 M, pH 7.4) and homogenized. The homogenate was centrifuged (10 min; 2,500 g, 4C°) to remove nuclei and debris, and then synaptosomes were isolated from the supernatant by centrifugation (20 min; 9,500 g, 4C°). The P2 pellet was then resuspended in 7 mL of Kreb's solution (mM: NaCl 118.5, KCl 4.7, $CaCl_2$ 1.2, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10; gassed with 95% O_2 and 5% CO_2) containing ascorbic acid (0.05 mM) and disodium EDTA (0.03 mM) to prevent [^{3}H]-DA degradation.

2.1.3. K^+-evoked [^{3}H]-DA and glutamate release

After the preparation, synaptosomes were incubated (37C°, 20 min) in the presence of 50 nM [^{3}H]-DA. At the end of this period, identical aliquots of synaptosomal suspension were distributed on microporous filters (0.5 mL/filter), placed at the bottom of a set of parallel superfusion chambers maintained at 37C° and perfused with aerated (95% O_2/5% CO_2) Krebs solution (0.3 mL/min).

After 30 min of superfusion, to equilibrate the system, 5 min fractions were collected from the 30th to the 75th minute (nine samples). After the collection of three basal samples, synaptosomes were depolarized with 15 mM K^+ (substituting for an equimolar concentration of NaCl) for 90 s. When required, cocaine (0.1-100 nM), quinpirole (10 nM-1µM; Sigma–Aldrich, USA), BD1063 (10-100 nM, Tocris Bioscience, UK) were added, alone or in combination, concomitantly with the depolarizing stimulus. At the end of the experiment, the radioactivity of the samples and filters was determined by liquid scintillation spectrometry. Aliquots of 100 µL were taken from each sample to measure endogenous glutamate levels.

2.1.4. Glutamate analysis

Glutamate was measured by HPLC with fluorimetric detection. Briefly, 25 µl were transferred into glass microvials and placed in a temperature-controlled (4°C) Triathlon autosampler (Spark Holland, Emmen, The Netherlands). Thirty µl of o-phthaldialdehyde/mercaptoethanol reagent were added to each sample, and 30 µl of the mixture were injected onto a Chromsep analytical column (3 mm inner diameter, 10 cm length; Chrompack, Middelburg, The Netherlands). The column was eluted at a flow rate of 0.48 ml/min (Beckman 125 pump; Beckman Instruments, Fullerton, CA, USA) with a mobile phase containing 0.1 M sodium acetate, 10% methanol and 2.2% tetrahydrofuran (pH 6.5). Glutamate was detected by means of a Jasco fluorescence spectrophotometer FP-2020 Plus (Jasco, Tokyo, Japan). The retention time of glutamate was ~3.5.

2.2. Luciferase reporter gene assay

2.2.1. Plasmid constructs, cell culture and transfection

The constructs presented herein were made using standard molecular biology as described previously [20]. HEK293T cells were grown and transiently transfected as depicted by Borroto-Escuela et al. [21,22].

*2.2.2. Luciferase reporter gene ass*ay

A dual luciferase reporter assay [22] has been used to indirectly detect variations of cAMP levels in transiently transfected cell lines treated with different compounds in a range of concentrations (typically 0.1 nM to 1 µM). For luciferase assays, 24 hours before transfection, cells were seeded at a density of 1 x 10^6 cells/well in 6-well dishes and transfected with Fugene (Promega, Stockholm, Sweden). Cells were co-transfected with plasmids corresponding to the four constructs as follows (per 6-well): 2 µg firefly luciferase-encoding experimental plasmid (pGL4-CRE-luc2p; Promega,

Stockholm, Sweden), 1 µg of D2R plus σ1R expression vectors and 0.5 µg Rluc-encoding internal control plasmid (phRG-B; Promega). Approximately 46 hours post transfection, cells were incubated with appropriate ligands and harvested with passive lysis buffer (Promega, Stockholm, Sweden). The luciferase activity of cell extracts was determined using the Dual-Luciferase® Reporter (DLR™) Assay System according to the manufacturer's protocol Promega, Stockholm, Sweden) in a POLAR star Optima plate reader (BMG Labtechnologies, Offenburg, Germany) using a 535 nm filter with a 30-nm bandwidth. Firefly luciferase was measured as firefly luciferase luminescence over a 15 s reaction period. The luciferase values were normalized against Rluc luminescence values (Luc/Rluc ratio). Chemicals used for the gene reporter assays (Raclopride, quinpirole, forskolin) were purchased from Tocris (UK) and (cocaine-HCL) Sigma Aldrich (Germany).

2.3. Data evaluation and statistical analysis

2.3.1. Synaptosomes

[^{3}H]-DA and glutamate release assays were carried out in duplicate and data are expressed as mean ± SEM of independent experiments.

The amount of radioactivity released into each fraction was expressed as a percentage of the total synaptosomal tritium present at the start of the respective collection period (fractional release). K^+-evoked [^{3}H]-DA overflow was calculated as net extra-outflow (NER) by subtracting the basal [^{3}H] release (determined by interpolation of the outflow measured 5 min before and 10 min after the onset of the stimulation) from the total tritium released during the 90 s stimulation and subsequent 8.5 min washout (i.e., two samples). This difference was calculated as a percentage of the total tissue tritium content at the onset of stimulation (fractional rate net extra-outflow). The effect of treatments was expressed as the percent ratio of the depolarization-evoked neurotransmitter overflow calculated in the presence of the drug versus that obtained in control group, i.e. K^+-evoked [^{3}H]-DA release measured in untreated synaptosomes, always assayed in parallel.

Spontaneous glutamate levels in each sample were expressed in nmol/min/g of protein [23]. Protein was determined according to Bradford [24]. The effects of K^+ stimulation on endogenous glutamate levels during the third fraction were reported and expressed as percentage changes of basal values, as calculated by the means of the two fractions collected prior to treatment [23].

Statistical analysis was performed using ANOVA followed by Newman–Keuls test for multiple comparisons. Statistical significance was assumed for $P<0.05$.

2.3.2. Cell experiments

The number of samples (n) in each experimental condition is indicated in figure legends. All data were analyzed using GraphPad PRISM 4.0 (GraphPad Software, USA). Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison post-test.

3. Results

3.1. Effects of quinpirole on K^+-evoked [^{3}H]-DA and glutamate release

As expected [11,25-27], the DA D_2-likeR agonist quinpirole, at 100 nM and 1 μM, caused a concentration-dependent reduction of the K^+ (15 mM; 90s)-evoked [^{3}H]-DA release from rat striatal synaptosomes. However, it was ineffective at the lower 10 nM concentration (Fig. 1A). Similar results were obtained when evaluating the effect of quinpirole on K^+-evoked glutamate release (Fig. 1B). The effects of quinpirole (100 nM) on K^+ (15 mM; 90s)-evoked [^{3}H]-DA and glutamate release from rat striatal synaptosomes were prevented by the DA D_2-likeR antagonist (S)-(-)-sulpiride (100 nM), by itself ineffective (Fig. 1).

At the concentration tested, quinpirole did not affect spontaneous [^{3}H]-DA and glutamate release from rat striatal synaptosomes (Table 1).

3.2. Effects of BD1063 on K^+-evoked [^{3}H]-DA and glutamate release

The σ_1R antagonist BD1063 (1-100 nM) did not influence spontaneous (Table 1) and K^+ (15 mM; 90s)-evoked [^{3}H]-DA and glutamate release from rat striatal synaptosomes (Fig. 2).

3.3. Effects of quinpirole in combination with BD1063 (1-100 nM) on K^+-evoked [^{3}H]-DA and glutamate release

3.3.1. 10 nM of quinpirole

In the presence of BD1063 (100 nM), quinpirole at a concentration by itself ineffective (10 nM), significantly decreased K^+-evoked [^{3}H]-DA, but not glutamate, release from rat striatal synaptosomes (Fig. 3). This effect was not observed when quinpirole was given with lower BD1063 concentrations (1 and 10 nM). All treatments did not affect spontaneous [^{3}H]-DA and glutamate release from rat striatal synaptosomes (Table 1).

3.3.2. 100 nM of quinpirole

As shown in Fig. 4, BD1063 (100 nM) significantly amplified the quinpirole (100 nM)-induced reduction of K^+-evoked [^{3}H]-DA, but not glutamate, release from rat striatal synaptosomes. At the lower 1 and 10 nM concentrations, BD1063 was ineffective. All treatments did not affect spontaneous [^{3}H]-DA and glutamate release from rat striatal synaptosomes (Table 1).

3.4. Effects of cocaine (0.1-100 nM) on quinpirole-induced reduction of K^+-evoked glutamate release

We previously reported that cocaine significantly enhanced the quinpirole (100 nM)-induced decrease of K^+-evoked [^{3}H]-DA release from rat striatal synaptosomes at 1 and 10 nM, whereas it was ineffective at 0.1 and 100 nM (Ferraro et al., 2010). As shown in Fig. 5, cocaine (10 nM) significantly amplified the quinpirole (100 nM)-induced decrease of K^+-evoked glutamate release from rat striatal synaptosomes. However, it was ineffective at lower concentrations (0.1 and 1 nM). At the concentration tested, cocaine by itself did not affect spontaneous glutamate release from rat striatal synaptosomes (Table 1).

3.5. Effects of cocaine (1 and 10 nM) in combination with BD1063 (1-10 nM) on the quinpirole (100 nM)-induced reduction of K^+-evoked [^{3}H]-DA and glutamate release

In the presence of BD1063 (10 nM), cocaine failed to amplify the quinpirole (100 nM)-induced reduction of K^+-evoked [^{3}H]-DA and glutamate release (Fig. 6). At the lower 1 nM concentration, the σ1R antagonist failed to counteract the effects of cocaine on quinpirole (100 nM)-induced reduction of K^+-evoked [^{3}H]-DA and glutamate release.

All treatments did not affect spontaneous [^{3}H]-DA and glutamate release from rat striatal synaptosomes (Table 1).

3.6. Effects of cocaine on the D_2-like agonist quinpirole induced inhibition of the AC-PKA-pCREB signaling cascade in σ_1R and D_{2L}R cotransfected and D_{2L} singly transfected HEK293T cells.

In cotransfected σ_1R and D_{2L}R HEK293T cells, quinpirole had a reduced potency to inhibit the CREB signal versus D_{2L}R singly transfected cells (Figure 7A). In the presence of cocaine (100 nM), the potency of the D_2-like agonist to inhibit the CREB signal in the cotransfected cells was restored (Figure 7A and 7B). In D_{2L} singly transfected cells cocaine at 100 nM and 10 µM exerted no modulatory effects on the inhibitory potency of quinpirole to bring down the CREB signal (Figure 7 C). In these cells cocaine alone at 100 nM, 1 µM and 10 µM also lacked effects on the CREB signal (*data not shown*).

4. Discussion

One interesting finding of the current paper was the demonstration that the σ_1R antagonist BD1063 at 100 nM could significantly enhance the inhibitory effects of the D_2-like agonist quinpirole (100 nM) on the K^+-evoked [^{3}H]-DA release from striatal synaptosomes. This was not true for the quinpirole induced inhibition of K^+-evoked glutamate release from striatal synaptosomes. At this and lower concentrations BD1063 alone failed to change K^+-evoked [^{3}H]-DA and glutamate release. This finding is in line with the demonstration that the σ_1R agonist PRE084 induces a significant decrease in the ability of D_2 receptors to signal through Gi [16]. A possible mechanism may be that both the D_2 autoreceptor and possibly the σ_1R control the voltage dependent calcium 1.3 (Ca_v 1.3) channels in the striatal DA terminals. These calcium channels are known to be inhibited by postjunctional D_2R [28] and D_2 autoreceptors [29] and possibly interact with σ_1Rs [30]. In the striato-pallidal GABA neurons, the D_2R upon agonist activation reduces L-type Ca_v 1.3 channel currents via the $\beta\gamma$ dimers released from Gi/o which turns on PLC and increases IP_3-calcineurin signalling. The dephosphorylation of this Ca_v channel leads to the closure of these channels and inhibition of firing of these neurons [28]. Similar events may take place in the striatal DA terminals involving the D_2 autoreceptors [29] and in the cortical/thalamic-striatal glutamate nerve terminal.

The σ_1R and the Ca_v 1.3 channel are proposed to be part of the same D_2 heteroreceptor complex in which D_2R activation leads to inhibition of the Ca_v 1.3 channel currents, while instead a σ_1R agonist, possibly endogenously released following K^+-evoked depolarization, may enhance them. Such a concept can explain the enhancement by the σ_1R antagonist BD1063 of quinpirole induced inhibition of K^+-evoked DA release. However, such a heteroreceptor complex remains to be demonstrated and should be less developed in the striatal glutamate terminals where such an enhancement by BD1063 of D_2 agonist inhibition of K^+-evoked glutamate release was not observed.

The major results were obtained in the cocaine experiments. Already at 1 and 10 nM of cocaine, it enhanced the inhibitory effects of quinpirole (100 nM) on K^+-evoked striatal [^{3}H]-DA release supporting previous results that cocaine in nanomolar concentrations can enhance Gi/o-mediated D_2R signaling by direct actions on the D_2 heteroreceptor complexes [10]. This is in apparent contrast to the results obtained with high (*i.e.* µM) concentrations of cocaine which lead to reduced D_2 signaling in the D_2R-σ_1R heterocomplexes [16]. Although other possibilities cannot be ruled out (*i.e.* the use of synaptosomes from rat, instead of mouse striatum) this discrepancy may be due to the different concentration of cocaine used in the current vs the Navarro et al. studies. In fact, it could be postulated that the allosteric receptor-receptor interactions in D_2-σ_1R heterocomplexes

change as the cocaine concentrations become reduced. It seems possible that cocaine in the high affinity range targets the σ_1R protomers in a different way leading to altered allosteric receptor-receptor interactions with enhancement of D_2R signaling. This was in fact demonstrated in the study on the HEK293T cells cotransfected with D_2 and σ_1R. Using the CREB gene reporter assay, cocaine at 100 nM was found to shift the concentration response curve to the left with a marked reduction of the IC_{50} value. This response was abolished when only the D_2 receptor was transfected. Such an enhancement of D_2R signaling may develop also over the Ca_v 1.3 channels mediated through release of βγ dimers as indicated also from the DA release experiments on striatal DA synaptosomes dependent on calcium channel signaling.

Of high interest was the demonstration in these cocaine experiments on [^{3}H]-DA release that the σ_1R receptor antagonist BD1063, at nM concentrations, counteracted the enhanced cocaine-quinpirole interactions. Thus, it seems possible that this is mediated by the altered allosteric receptor-receptor interactions induced by cocaine targeting the σ_1R protomer in the postulated D_2R-σ_1R- Ca_v 1.3 channel complex. Similar events were found to take place in the K^+-evoked glutamate release experiments under the influence of cocaine but only at 10 nM, thus validating the preferential role of these effects on striatal DA terminals. The possible relevance of this finding is underlined by the evidence that antagonism of σ_1R receptors attenuates cocaine-induced convulsions, acute locomotor stimulation, locomotor sensitization as well as cocaine-induced conditioned place preference [31,32]. There exists evidence for a specific interaction between σ1R and DAT, and that the transmembrane domain of σ1R likely mediated this interaction [33]. According to the current results it seems possible that the σ1R antagonist BD1063 at 10nM modulated the DA transporter through targeting a putative σ1R-D2-DAT heteroreceptor complex located on the DA nerve terminals.

5. Conclusions

Taken together, the current results on [^{3}H]-DA and glutamate release from striatal synaptosomes induced by K^+ with or without the influence of cocaine give indications of the possible existence of D2-σ_1R-Ca_v 1.3 heteroreceptor complexes in the striatal DA and glutamate nerve terminals. Cocaine in nanomolar concentrations appears to alter the allosteric receptor-receptor interactions in such complexes leading to enhancement of Gi/o mediated D_2R signalling. This can involve changes in the pharmacology of the σ_1Rs due to allosteric changes induced by cocaine through targeting the sigma1 receptors.

Disclosure

None.

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References

[1] G. Di Chiara, A. Imperato, Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats, Proc. Natl. Acad. Sci. USA 85 (1988) 5274-5278.

[2] G. F. Koob, F. E. Bloom, Cellular and molecular mechanisms of drug dependence, Science 242 (1988) 715-723.

[3] P. W. Kalivas, N. D. Volkow, The neural basis of addiction: a pathology of motivation and choice, Am. J. Psychiatry 162 (2005) 1403-1413.

[4] C. De Mei, M. Ramos, C. Iitaka, E. Borrelli, Getting specialized: presynaptic and postsynaptic dopamine D2 receptors, Curr. Opin. Pharmacol. 9 (2009) 53-58.

[5] M. C. Ritz, R. J. Lamb, S. R. Goldberg, M. J. Kuhar, Cocaine receptors on dopamine transporters are related to self-administration of cocaine, Science 237 (1987) 1219-1223.

[6] B. Giros, M. Jaber, S. R. Jones, R. M. Wightman, M. G. Caron, Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter, Nature 379 (1996) 606-612.

[7] R. Chen, M. R. Tilley, H. Wei, F. Zhou, F. M. Zhou, S. Ching, N. Quan, R. L. Stephens, E. R. Hill, T. Nottoli, D. D. Han, H. H. Gu, Abolished cocaine reward in mice with a cocaine-insensitive dopamine transporter, Proc. Natl. Acad. Sci. USA 103 (2006) 9333-9338.

[8] D. Marcellino, G. Navarro, K. Sahlholm, J. Nilsson, L. F. Agnati, E. I. Canela, C. Lluís, P. Århem, R. Franco, K. Fuxe, Cocaine produces D2R-mediated conformational changes in the adenosine A(2A)R-dopamine D2R heteromer, Biochem. Biophys. Res. Commun. 394 (2010) 988-992.

[9] S. Genedani, C. Carone, D. Guidolin, M. Filaferro, D. Marcellino, K. Fuxe, L. F. Agnati, Differential sensitivity of A2A and especially D2 receptor trafficking to cocaine compared with lipid rafts in cotransfected CHO cell lines. Novel actions of cocaine independent of the DA transporter, J. Mol. Neurosci. 4 (2010) 347-357.

[10] L. Ferraro, M. Frankowska, D. Marcellino, M. Zaniewska, S. Beggiato, M. Filip, M. C. Tomasini, T. Antonelli, S. Tanganelli, K. Fuxe, A novel mechanism of cocaine to enhance dopamine d2-like receptor mediated neurochemical and behavioral effects. An in vivo and in vitro study. Neuropsychopharmacology 37 (2012) 1856-1866.

[11] L. Ferraro, S. Beggiato, D. Marcellino, M. Frankowska, M. Filip, L. F. Agnati, T. Antonelli, M. C. Tomasini, S. Tanganelli, K. Fuxe, Nanomolar concentrations of cocaine enhance D2-like agonist-induced inhibition of the K^+-evoked [^{3}H]-dopamine release from rat striatal synaptosomes: a novel action of cocaine, J. Neural Transm. 117 (2010) 593-597.

[12] F. Rouge-Pont, A. Usiello, M. Benoit-Marand, F. Gonon, P. V. Piazza, E. Borrelli, Changes in extracellular dopamine induced by morphine and cocaine: crucial control by D2 receptors, J. Neurosci. 22 (2002) 3293-3301.

[13] J. W. Dalley, B. J. Everitt, Dopamine receptors in the learning, memory and drug reward circuitry, Semin. Cell Dev. Biol. 20 (2009) 403-410.

[14] J. W. Dalley, T. D. Fryer, F. I. Aigbirhio, L. Brichard, H. K. Richards, Y.T. Hong, J.C. Baron, B. J. Everitt, T. W. Robbins, Modelling human drug abuse and addiction with dedicated small animal positron emission tomography, Neuropharmacology 56, Suppl 1 (2009) 9-17.

[15] G. Alonso, V. Phan, I. Guillemain, M. Saunier, A. Legrand, M. Anoal, T. Maurice, Immunocytochemical localization of the sigma(1) receptor in the adult rat central nervous system, Neuroscience 97 (2000) 155-170.

[16] G. Navarro, E. Moreno, J. Bonaventura, M. Brugarolas, D. Farré, D. Aguinaga, J. Mallol, A. Cortés, V. Casadó, C. Lluís, S. Ferrè, R. Franco, E. Canela, P. J. McCormick, Cocaine inhibits dopamine D2 receptor signaling via sigma-1-D2 receptor heteromers, PLoS One 8 (2013) e61245.

[17] T. Maurice, R. Martin-Fardon, P. Romieu, R. R. Matsumoto, Sigma(1) receptor antagonists represent a new strategy against cocaine addiction and toxicity, Neurosci. Biobehav. Rev. 26 (2002) 499-527.

[18] R. R. Matsumoto, L. Nguyen, N. Kaushal, M. J. Robson, Sigma (σ) receptors as potential therapeutic targets to mitigate psychostimulant effects, Adv. Pharmacol. 69 (2014) 323-386.

[19] R. R. Matsumoto, K. A. McCracken, B. Pouw, Y. Zhang, W. D. Bowen, Involvement of sigma receptors in the behavioral effects of cocaine: evidence from novel ligands and antisense oligodeoxynucleotides. Neuropharmacology 42 (2002) 1043-1055.

[20] D. O. Borroto-Escuela, A. Ravani, A. O. Tarakanov, I. Brito, M. Narvaez, W. Romero-Fernandez, F. Corrales, L. F. Agnati, S. Tanganelli, L. Ferraro, K. Fuxe, Dopamine D2 receptor signaling dynamics of dopamine D2-neurotensin 1 receptor heteromers. Biochem. Biophys. Res. Commun. 435 (2013) 140-146.

[21] D. O. Borroto-Escuela, M. Narvaez, D. Marcellino, C. Parrado, J. A. Narvaez, A. O. Tarakanov, L. F. Agnati, Z. Diaz-Cabiale, K. Fuxe, Galanin receptor-1 modulates 5-hydroxtryptamine-1A signaling via heterodimerization, Biochem. Biophys. Res. Commun. 393 (2010) 767-772.

[22] D. O. Borroto-Escuela, W. Romero-Fernandez, A. O. Tarakanov, D. Marcellino, F. Ciruela, L. F. Agnati, K. Fuxe, Dopamine D2 and 5-hydroxytryptamine 5-HT((2)A) receptors assemble into functionally interacting heteromers, Biochem. Biophys. Res. Commun. 401 (2010) 605-610.

[23] A. Giuliani, S. Beggiato, V. A. Baldassarro, C. Mangano, L. Giardino, B. P. Imbimbo, T. Antonelli, L. Calzà, L. Ferraro, CHF5074 restores visual memory ability and pre-synaptic cortical acetylcholine release in pre-plaque Tg2576 mice, J. Neurochem. 124 (2013) 613-620.

[24] M. M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248-254.

[25] G. Maura, R. Carbone, M. Raiteri, Aspartate-releasing nerve terminals in rat striatum possess D-2 dopamine receptors mediating inhibition of release, J. Pharmacol. Exp. Ther. 251 (1989) 1142-1146.

[26] M. L'hirondel, A. Chéramy, G. Godeheu, F. Artaud, A. Saiardi, E. Borrelli, J. Glowinski, Lack of autoreceptor-mediated inhibitory control of dopamine release in striatal synaptosomes of D2 receptor-deficient mice, Brain Res. 792 (1998) 253-262.

[27] M. A. Yousfi-Alaoui, S. Hospital, A. Garcia-Sanz, A. Badia, M. V. Clos, Presynaptic modulation of K+-evoked [3H]dopamine release in striatal and frontal cortical synaptosomes of normotensive and spontaneous-hypertensive rats, Neurochem. Res. 26 (2001) 1271-1275.

[28] S. Hernandez-Lopez, T. Tkatch, E. Perez-Garci, E. Galarraga, J. Bargas, H. Hamm, D. J. Surmeier, D2 dopamine receptors in striatal medium spiny neurons reduce L-type Ca2+ currents and excitability via a novel PLC[beta]1-IP3-calcineurin-signaling cascade. J Neurosci. 20 (2000) 8987-8995.

[29] E. Dragicevic, C. Poetschke, J. Duda, F. Schlaudraff, S. Lammel, J. Schiemann, M. Fauler, A. Hetzel, M. Watanabe, R. Lujan, R. C Malenka., J. Striessnig, B. Liss, Cav1.3 channels control D2-autoreceptor responses via NCS-1 in substantia nigra dopamine neurons. Brain 137 (2014) 2287-2302.

[30] D. Crottès, H. Guizouarn, P. Martin, F. Borgese, O. Soriani, The sigma-1 receptor: a regulator of cancer cell electrical plasticity?, Front. Physiol. 4 (2013) 175.

[31] R. R. Matsumoto, K. A. McCracken, B. Pouw, Y. Zhang, W. D. Bowen, Involvement of sigma receptors in the behavioral effects of cocaine: evidence from novel ligands and antisense oligodeoxynucleotides, Neuropharmacology 42 (2002) 1043-1055.

[32] R. R. Matsumoto, Y. Liu, M. Lerner, E. W. Howard, D. J. Brackett, Sigma receptors: potential medications development target for anti-cocaine agents, Eur. J. Pharmacol. 469 (2003) 1-12.

[33] W. C. Hong, H. Yano, T. Hiranita, F. T. Chin, C. R. McCurdy, T. P. Su, S. G. Amara, J. L. Katz, The sigma-1 receptor modulates dopamine transporter conformation and cocaine binding and may thereby potentiate cocaine self-administration in rats, J. Biol. Chem. 292 (2017) 11250-11261.

Figure legends

Figure 1. Effects of quinpirole (Quin; 10 nM-1 μM) on K^+-evoked [^{3}H]-DA (*panel A*) and glutamate (*panel B*) release from rat striatal synaptosomes. The dopamine D_2-like receptor agonist was added concomitantly with the depolarizing stimulus (15 mM K^+, 90 s). The same volume of vehicle (Kreb's solution) was combined to the depolarizing stimulus in the control groups. The effect of the treatments on K^+-evoked [^{3}H]-DA and glutamate release is expressed as percent of control values ([^{3}H]-DA = 100 ± 2%; glutamate = 100 ± 4%, n = 19; indicated by dashed lines) *i.e.* K^+-evoked [^{3}H]-DA and glutamate release measured in untreated synaptosomes, always assayed in parallel. Each treatment bar represents the mean ± SEM of 6-7 determinations run in duplicate. **$p<0.01$ significantly different from the respective control and Quin (10 nM) groups; °$p<0.05$ significantly different from the respective Quin (100 nM) group, according to ANOVA followed by Newman–Keuls test for multiple comparisons.

Figure 2. Effects of the σ_1 receptor antagonist BD1063 (BD; 1-100 nM) on K^+-evoked [^{3}H]-DA (*panel A*) and glutamate (*panel B*) release from rat striatal synaptosomes. BD1063 was added concomitantly with the depolarizing stimulus (15 mM K^+, 90 s). The same volume of vehicle (Kreb's solution) was combined to the depolarizing stimulus in the control groups. The effect of the treatments on K^+-evoked [^{3}H]-DA and glutamate release is expressed as percent of control values ([^{3}H]-DA = 100 ± 3%; glutamate = 100 ± 3%, n = 15; indicated by dashed lines) *i.e.* K^+-evoked [^{3}H]-DA and glutamate release measured in untreated synaptosomes, always assayed in parallel. Each treatment bar represents the mean ± SEM of 5-7 determinations run in duplicate.

Figure 3. Effects of the σ_1 receptor antagonist BD1063 (BD; 1-100 nM) on the quinpirole (Quin; 10 nM)-induced modification of K^+-evoked [^{3}H]-DA (*panel A*) and glutamate (*panel B*) release from rat striatal synaptosomes. The drugs were added alone or in combination concomitantly with the depolarizing stimulus (15 mM K^+, 90 s). The same volume of vehicle (Kreb's solution) was combined to the depolarizing stimulus in the control groups. The effect of the treatments on K^+-evoked [^{3}H]-DA and glutamate release is expressed as percent of control values ([^{3}H]-DA = 100 ± 4%; glutamate = 100 ± 2%, n = 19; indicated by dashed lines) *i.e.* K^+-evoked [^{3}H]-DA and glutamate release measured in untreated synaptosomes, always assayed in parallel. Each treatment bar represents the mean ± SEM of 6-8 determinations run in duplicate. **$p<0.01$ significantly different from the respective control and other groups, according to ANOVA followed by Newman–Keuls test for multiple comparisons.

Figure 4. Effects of the σ_1 receptor antagonist BD1063 (BD; 1-100 nM) on the quinpirole (Quin; 100 nM)-induced inhibition of K^+-evoked [^{3}H]-DA (*panel A*) and glutamate (*panel B*) release from rat striatal synaptosomes. The drugs were added alone or in combination concomitantly with the depolarizing stimulus (15 mM K^+, 90 s). The same volume of vehicle (Kreb's solution) was combined to the depolarizing stimulus in the control groups. The effect of the treatments on K^+-evoked [^{3}H]-DA and glutamate release is expressed as percent of control values ([^{3}H]-DA = 100 ± 3%; glutamate = 100 ± 2%, n = 17; indicated by dashed lines) *i.e.* K^+-evoked [^{3}H]-DA and glutamate release measured in untreated synaptosomes, always assayed in parallel. Each treatment bar represents the mean ± SEM of 6-8 determinations run in duplicate. *$p<0.05$, **$p<0.01$ significantly different from the respective control group; °$p<0.05$ significantly different from the respective Quin (100 nM), Quin (100 nM) + BD (1 nM) as well as Quin (100 nM) + BD (10 nM) groups, according to ANOVA followed by Newman–Keuls test for multiple comparisons.

Figure 5. Effects of cocaine on the quinpirole (Quin; 100 nM)-induced inhibition of K^+-evoked glutamate release from rat striatal synaptosomes. The drugs were added alone or in combination concomitantly with the depolarizing stimulus (15 mM K^+, 90 s). The same volume of vehicle (Kreb's solution) was combined to the depolarizing stimulus in the control groups. The effect of the treatments on K^+-evoked glutamate release is expressed as percent of control values (100 ± 3%, n = 17; indicated by a dashed line) *i.e.* K^+-evoked glutamate release measured in untreated synaptosomes, always assayed in parallel. Each treatment bar represents the mean ± SEM of 6-8 determinations run in duplicate. **$p<0.01$ significantly different from control and cocaine alone groups; °$p<0.05$ significantly different from Quin, Quin + cocaine (0.1 nM) as well as Quin + cocaine (1 nM) groups, according to ANOVA followed by Newman–Keuls test for multiple comparisons.

Figure 6. Effects of the σ_1 receptor antagonist BD1063 (BD; 1 and 10 nM) on cocaine-provoked amplification of quinpirole (Quin; 100 nM)-induced reduction of K^+-evoked [^{3}H]-DA and glutamate release from rat striatal synaptosomes. The drugs were added alone or in combination concomitantly with the depolarizing stimulus (15 mM K^+, 90 s). The same volume of vehicle (Kreb's solution) was combined to the depolarizing stimulus in the control groups. The effect of the treatments on K^+-evoked [^{3}H]-DA and glutamate release is expressed as percent of control values ([^{3}H]-DA = 100 ± 4%; glutamate = 100 ± 3%, n = 19; indicated by dashed lines) *i.e.* K^+-evoked [^{3}H]-DA and glutamate release measured in untreated synaptosomes, always assayed in parallel.

Each treatment bar represents the mean ± SEM of 6-8 determinations run in duplicate. *$p<0.05$, **$p<0.01$ significantly different from the respective control and cocaine alone groups; °$p<0.05$ significantly different from the respective Quin alone group, according to ANOVA followed by Newman–Keuls test for multiple comparisons.

Figure 7. Quinpirole-induced $D_{2L}R$ activation in a forskolin-induced CRE-luciferase reporter gene assay. (*Panels A and B*) Concentration-response curves with quinpirole in σ_1R and D_2R co-transfected HEK293T cells in the absence (vehicle) or in presence of cocaine. HEK293T cells were transiently co-transfected with 2 µg firefly luciferase-encoding experimental plasmid (pGL4-CRE-luc2p), 1 µg of both (D_2R and σ_1R) expression vectors and 0.5 µg Renilla luciferase-encoding internal control plasmid (phRG-B). Forty-six hours after transfection, cells were treated for 30 min with 2 µM forskolin (sub-maximal concentration value), and quinpirole in either the absence or presence of cocaine 100 nM and the luciferase activity was measured after 4 hours. Luminescence emission is expressed as a percentage of the forskolin-induced maximal response value. The addition of cocaine (100 nM) shifted the curve to the left and the EC_{50} value became significantly reduced (*see panel A*, * $p<0.05$). In *panel A*, the negative logarithm of the EC_{50} value is given. (*Panel C*) Concentration-response curves with quinpirole in D_2R single transfected HEK293T cells, in the absence (vehicle) or in presence of cocaine. HEK293T cells were transiently transfected with 2 µg firefly luciferase-encoding experimental plasmid (pGL4-CRE-luc2p), 1 µg of both (D2R and pcDNA3.1+) expression vectors and 0.5 µg Renilla luciferase-encoding internal control plasmid (phRG-B). Forty-six hours after transfection, cells were treated for 30 min with 2 µM forskolin (sub-maximal concentration value), and quinpirole in either the absence or presence of cocaine 100 nM and 10 µM and the luciferase activity was measured after 4 hours. Luminescence emission is expressed as a percentage of the forskolin-induced maximal response value. The addition of cocaine (100 nM or 10 µM) did not produce a significant change in the EC_{50} value. The data in all cases represent the mean ± S.E.M. of 7 independent experiments performed in 8 replicates. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison post-test.

Figure 1

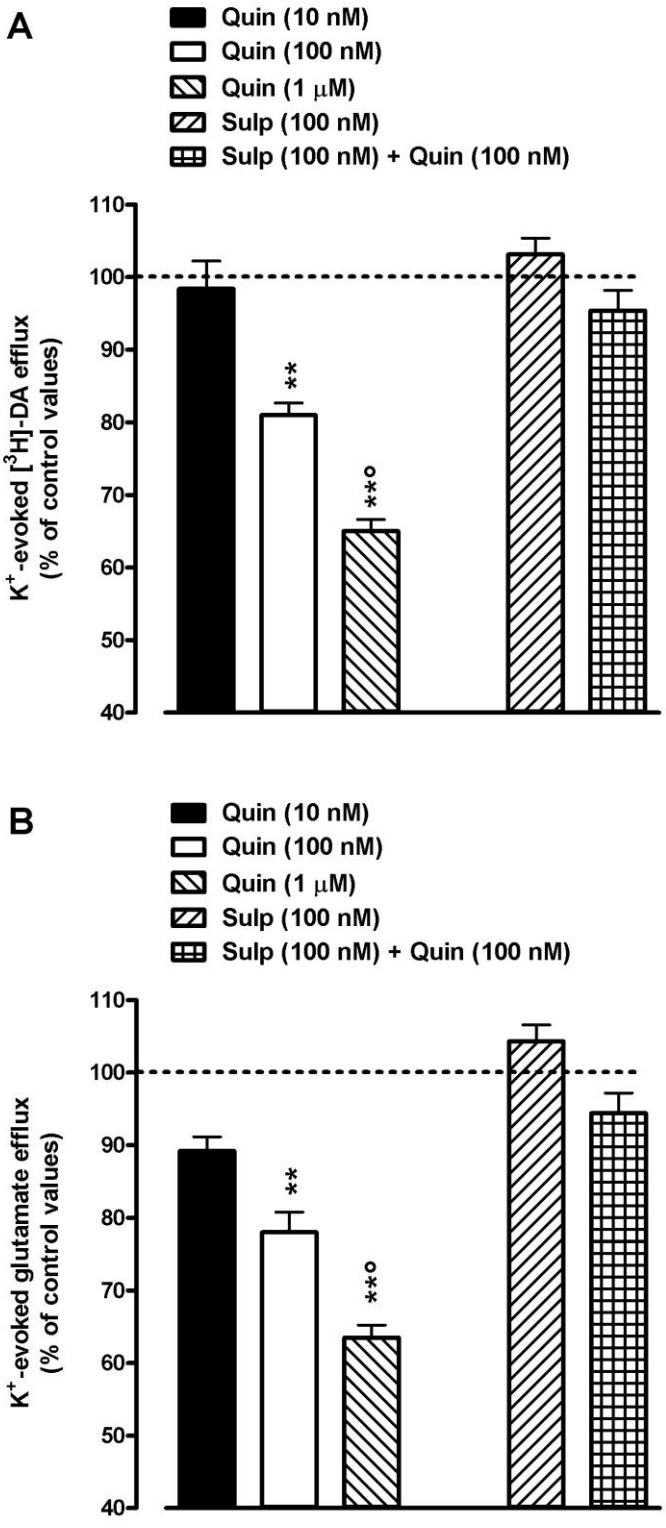

A
Quin (10 nM)
Quin (100 nM)
Quin (1 μM)
Sulp (100 nM)
Sulp (100 nM) + Quin (100 nM)
K+-evoked [3H]-DA efflux
(% of control values)
110
100
90
80
70
60
50
40
**
***
B
Quin (10 nM)
Quin (100 nM)
Quin (1 μM)
Sulp (100 nM)
Sulp (100 nM) + Quin (100 nM)
K+-evoked glutamate efflux
(% of control values)
110
100
90
80
70
60
50
40
**
***

Figure 2

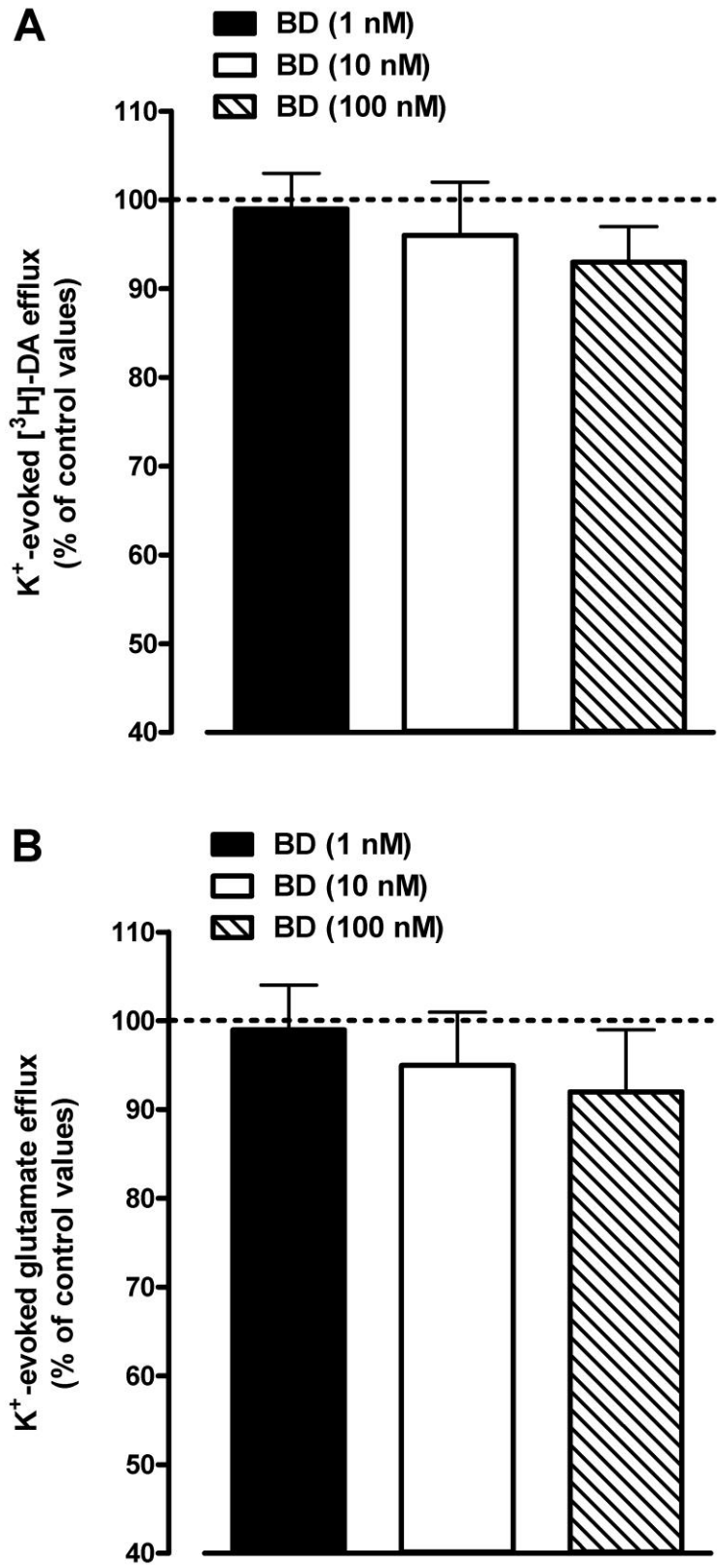

A
BD (1 nM)
BD (10 nM)
BD (100 nM)
110
100
90
80
70
60
50
40
K+-evoked [3H]-DA efflux
(% of control values)
B
BD (1 nM)
BD (10 nM)
BD (100 nM)
110
100
90
80
70
60
50
40
K+-evoked glutamate efflux
(% of control values)

Figure 3

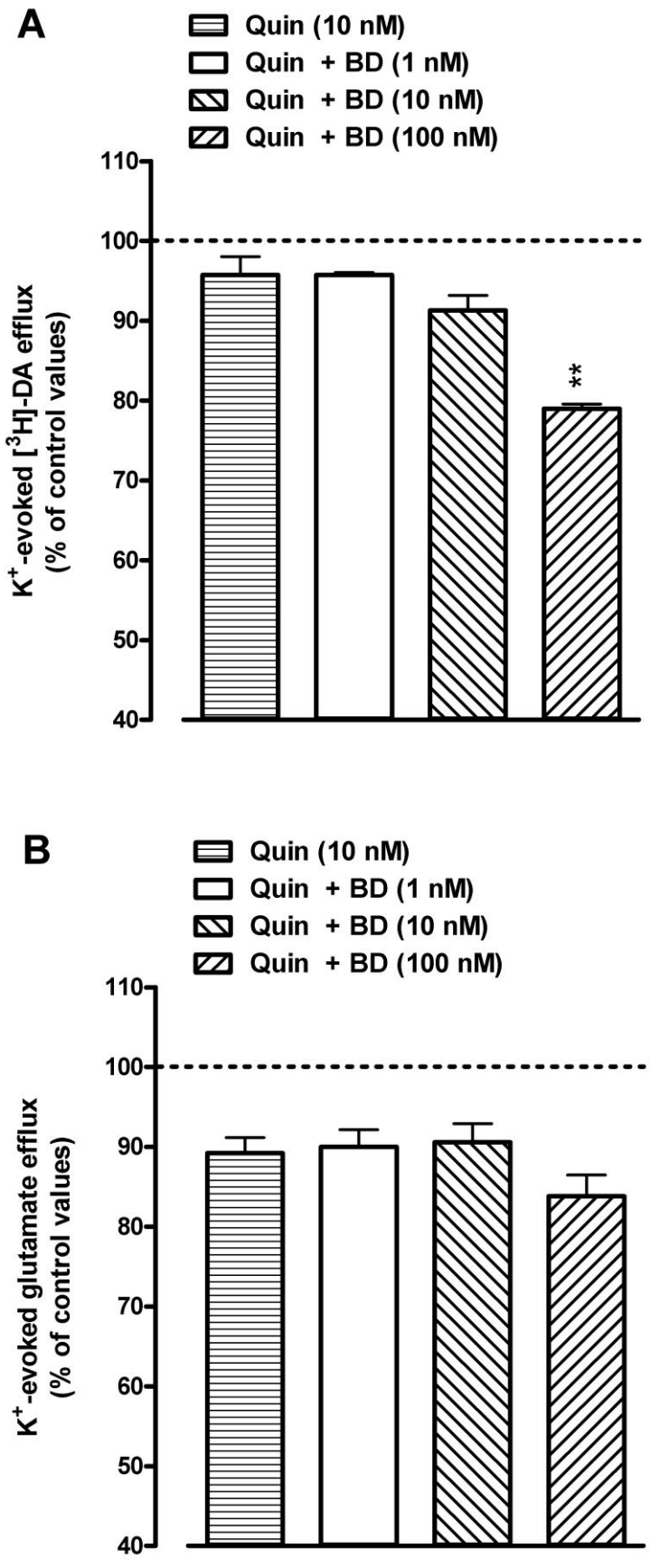

A
Quin (10 nM)
Quin + BD (1 nM)
Quin + BD (10 nM)
Quin + BD (100 nM)
K+-evoked [3H]-DA efflux
(% of control values)
110
100
90
80
70
60
50
40
**
B
Quin (10 nM)
Quin + BD (1 nM)
Quin + BD (10 nM)
Quin + BD (100 nM)
K+-evoked glutamate efflux
(% of control values)
110
100
90
80
70
60
50
40

Figure 4

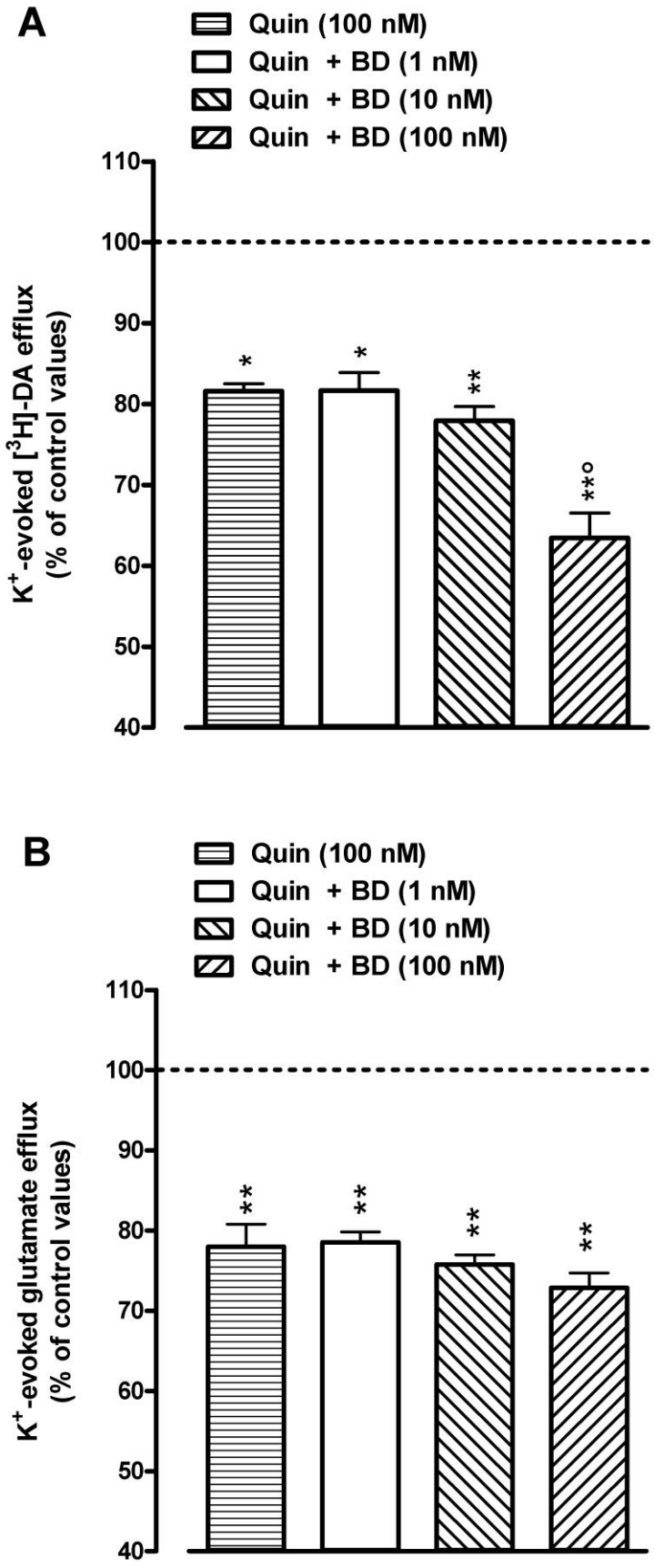

A
Quin (100 nM)
Quin + BD (1 nM)
Quin + BD (10 nM)
Quin + BD (100 nM)
K+-evoked [3H]-DA efflux
(% of control values)
110
100
90
80
70
60
50
40
*
*
**
**°
B
Quin (100 nM)
Quin + BD (1 nM)
Quin + BD (10 nM)
Quin + BD (100 nM)
K+-evoked glutamate efflux
(% of control values)
110
100
90
80
70
60
50
40
**
**
**
**

Figure 5

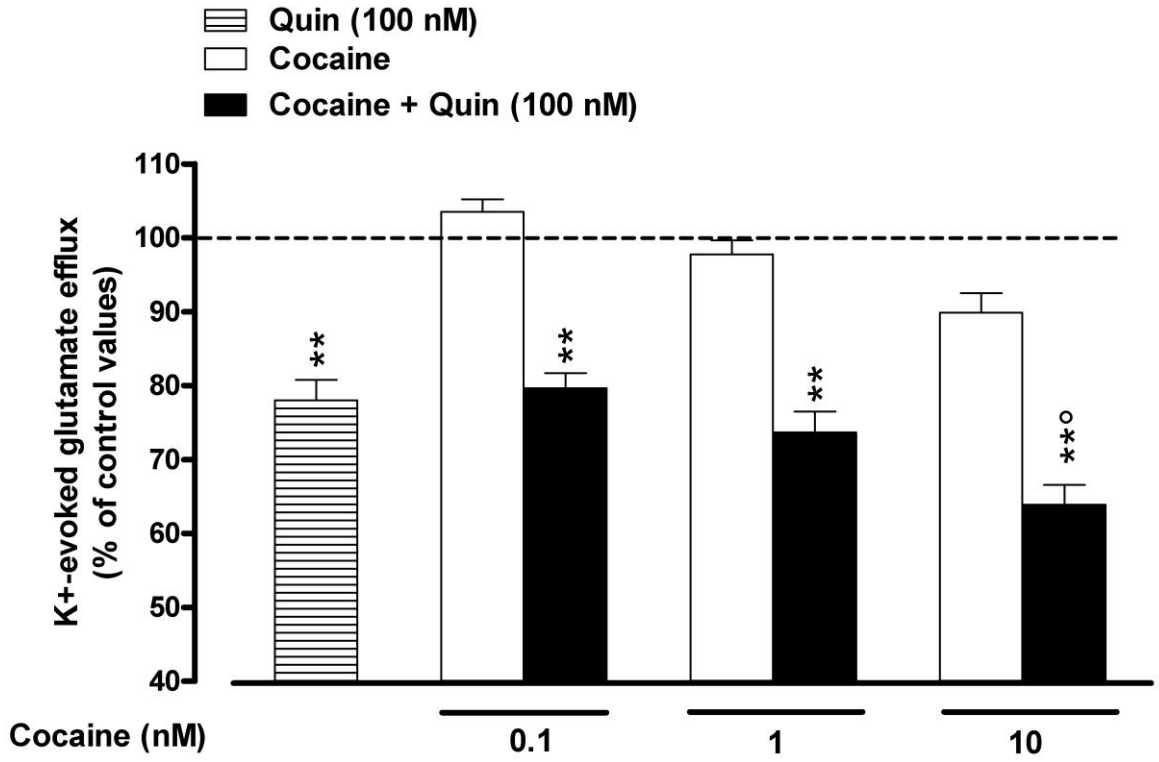
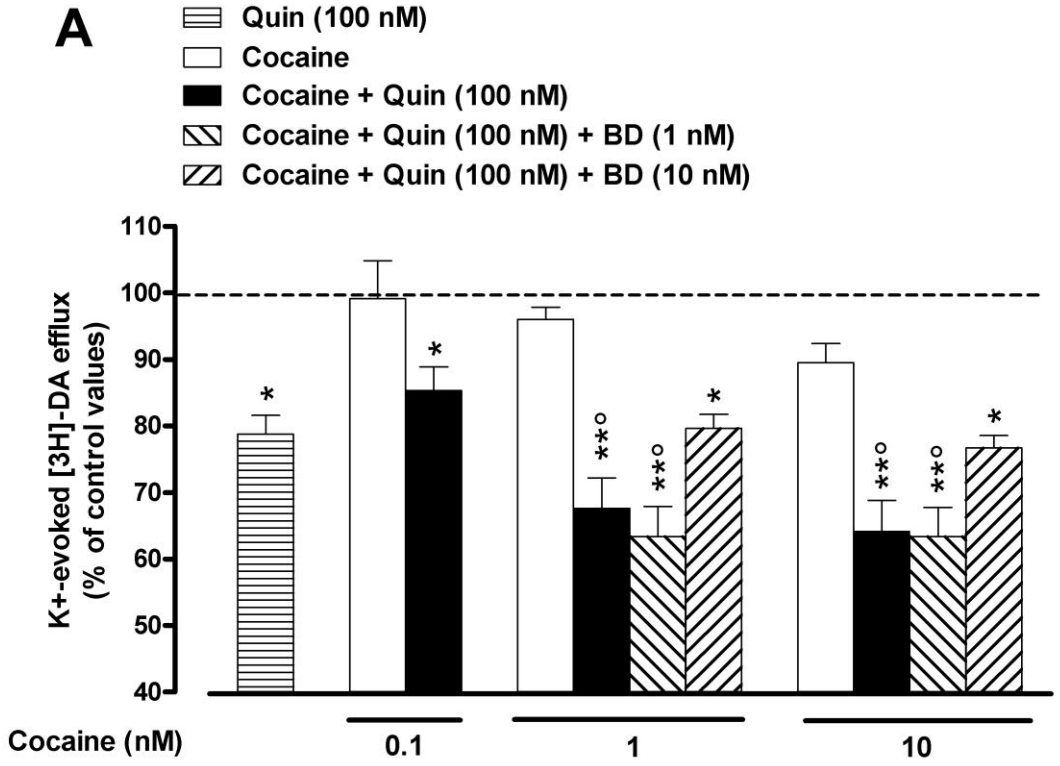

Quin (100 nM)
Cocaine
Cocaine + Quin (100 nM)
K+-evoked glutamate efflux
(% of control values)
110
100
90
80
70
60
50
40
**
**
**
**°
Cocaine (nM)
0.1
1
10

Figure 6


A
Quin (100 nM)
Cocaine
Cocaine + Quin (100 nM)
Cocaine + Quin (100 nM) + BD (1 nM)
Cocaine + Quin (100 nM) + BD (10 nM)
K+-evoked [3H]-DA efflux
(% of control values)
110
100
90
80
70
60
50
40
Cocaine (nM)
0.1
1
10

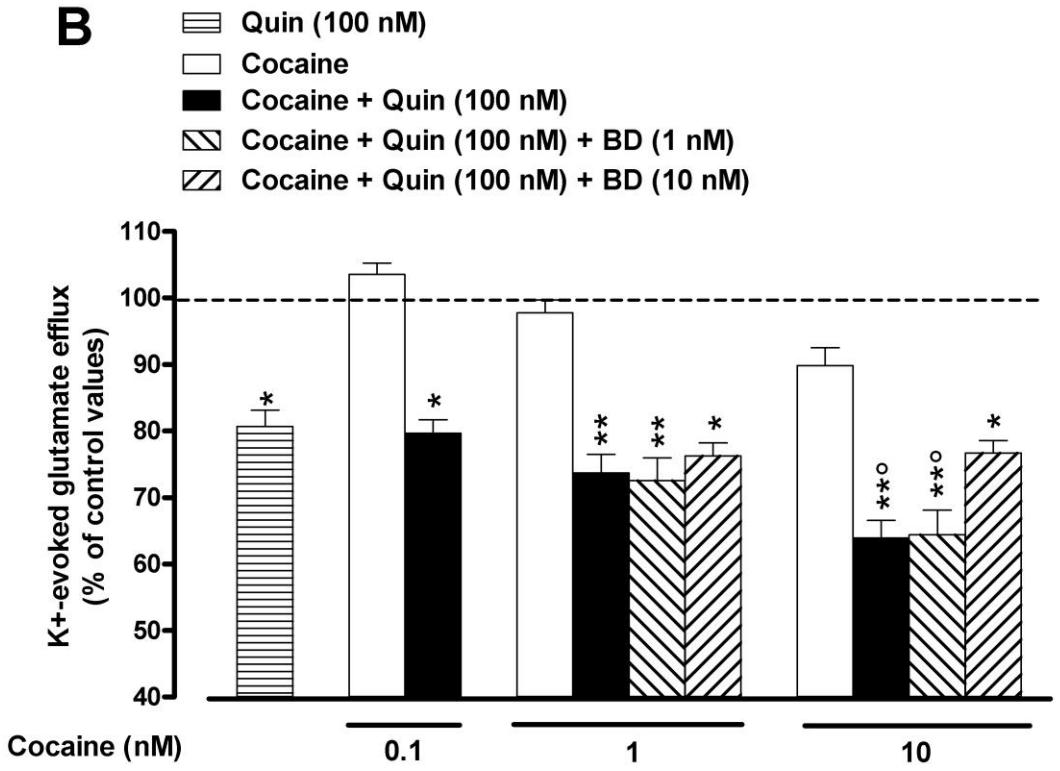

B
Quin (100 nM)
Cocaine
Cocaine + Quin (100 nM)
Cocaine + Quin (100 nM) + BD (1 nM)
Cocaine + Quin (100 nM) + BD (10 nM)
K+-evoked glutamate efflux
(% of control values)
110
100
90
80
70
60
50
40
Cocaine (nM)
0.1
1
10

Figure 7

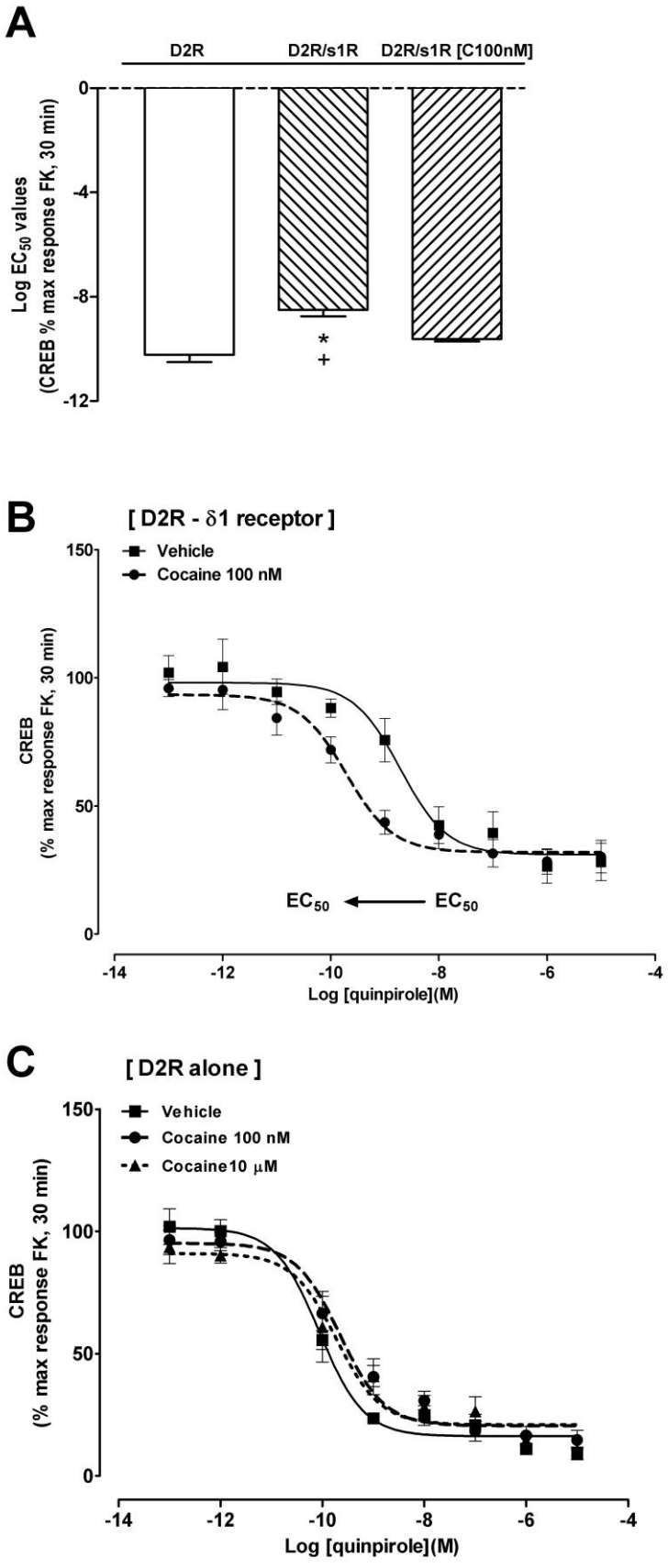

A
D2R
D2R/s1R
D2R/s1R [C100nM]
Log EC50 values
(CREB % max response FK, 30 min)
0
-4
-8
-12
*
+
B
[D2R - δ1 receptor]
Vehicle
Cocaine 100 nM
CREB
(% max response FK, 30 min)
150
100
50
0
EC50
EC50
-14
-12
-10
-8
-6
-4
Log [quinpirole](M)
C
[D2R alone]
Vehicle
Cocaine 100 nM
Cocaine10 μM
CREB
(% max response FK, 30 min)
150
100
50
0
-14
-12
-10
-8
-6
-4
Log [quinpirole](M)

Table 1. Effects of the treatments on spontaneous [^{3}H]-DA and glutamate release

Treatment(s)	[^{3}H]-DA	Glutamate
Control	-407 ± 205	-327 ± 152
Quin (10 nM)	-415 ± 189	-401 ± 188
Quin (100 nM)	-539 ± 227	-328 ± 153
Quin (1 µM)	-473 ± 199	-381 ± 177
Sul (100 nM)	-492 ± 257	-376 ± 181
Quin (1 µM) + Sul (100 nM)	-428 ± 224	-401 ± 172
BD (1 nM)	-389 ± 256	-412 ± 192
BD (10 nM)	-472 ± 234	-318 ± 127
BD (100 nM)	-438 ± 193	-402 ± 176
Quin (10 nM) + BD (1 nM)	-446 ± 257	-349 ± 131
Quin (10 nM) + BD (10 nM)	-459 ± 193	-423 ± 176
Quin (10 nM) + BD (100 nM)	-511 ± 271	-407 ± 185
Quin (100 nM) + BD (1 nM)	-485 ± 284	-400 ± 179
Quin (100 nM) + BD (10 nM)	-412 ± 182	-422 ± 194
Quin (100 nM) + BD (100 nM)	-449 ± 218	-359 ± 229
Cocaine (0.1 nM)	-455 ± 204	-392 ± 202
Cocaine (1 nM)	-507 ± 189	-327 ± 274
Cocaine (10 nM)	-477 ± 193	-381 ± 175
Cocaine (0.1 nM) + Quin (100 nM)	-436 ± 242	-444 ± 258
Cocaine (1 nM) + Quin (100 nM)	-485 ± 259	-391 ± 197
Cocaine (10 nM) + Quin (100 nM)	-504 ± 284	-353 ± 200
Cocaine (0.1 nM) + Quin (100 nM) + BD (1 nM)	-418 ± 277	-374 ± 199
Cocaine (0.1 nM) + Quin (100 nM) + BD (10 nM)	-487 ± 264	-359 ± 214
Cocaine (1 nM) + Quin (100 nM) + BD (1 nM)	-491 ± 283	-404 ± 162
Cocaine (1 nM) + Quin (100 nM) + BD (10 nM)	-421 ± 274	-337 ± 190
Cocaine (10 nM) + Quin (100 nM) + BD (1 nM)	-500 ± 244	-342 ± 205
Cocaine (10 nM) + Quin (100 nM) + BD (10 nM)	-438 ± 271	-339 ± 183

In synaptosomes from rat striatum, spontaneous [^{3}H]-DA and glutamate release tended to decrease during the collection period (from 30th to 75th minutes from the start of perfusion. The drugs were added alone or in combination after three basal samples have been collected. The same volume of vehicle (Kreb's solution) was added to the perfusion medium in the control groups. The results are expressed as the area created by the time-course curve (AUC), which was determined for each animal. Area values (overall effects) were calculated as percentages of changes in baseline value over time by using the trapezoidal rule. Each value represents the mean ± SEM of 5-8 determinations.