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α_2-Adrenergic control of dopamine overflow and metabolism in mouse striatum

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Abstract

The effects of the α_2-adrenoceptor drugs, medetomidine and atipamezole, on dopamine overflow evoked by low (6 Hz–10 s) and high (50 Hz–4 s) frequency electrical stimulation of the median forebrain bundle were studied in striatum of BALB/C mice anaesthetized with chloral hydrate with fast in vivo voltammetry techniques. The effects of these drugs on the basal concentrations of dopamine metabolites were also investigated by means of differential pulse voltammetry. Medetomidine dose dependently decreased dopamine overflow in nucleus accumbens in the dose range 5–100 μg/kg, s.c. This effect was seen only at low frequency stimulation and reached 85% at a dose of 100 μg/kg. Medetomidine also decreased the basal concentration of striatal homovanillic acid. This effect did not exceed 35%. Atipamezole antagonized the inhibitory effects of medetomidine on the dopamine overflow, but showed no effect itself. We suggest that α_2-adrenoceptors in dopaminergic terminal fields in the mouse striatum are involved in the regulation of dopamine release at physiological stimulation frequencies. © 1997 Elsevier Science B.V.

Keywords: Atipamezole; Medetomidine; Dopamine overflow; Dopamine metabolisms; Voltammetry, in vivo; Mouse

1. Introduction

There is an abundance of data showing that specific adrenoceptor agonists that do not themselves have any affinity for dopamine receptors nonetheless are capable of modulating dopaminergic neurotransmission (Anden and Grabowska, 1976; Waldmeier et al., 1982; MacDonald et al., 1988; Koulu et al., 1993). It has been suggested that noradrenergic neurons originating in the locus coeruleus exert a tonic excitatory influence on striatal and limbic dopaminergic neurons. Lesions of the locus coeruleus with neurotoxins (Lategan et al., 1990, 1992) decrease the turnover of dopamine in striatum, and lesions of the noradrenergic fibers innervating the ventral tegmental area decrease the turnover of dopamine in prefrontal cortex (Herve et al., 1982). The α_2-adrenoceptor agonist, clonidine, regularizes the firing rate of the dopamine cells in the ventral tegmental area (Grenhoff and Svensson, 1989). Since regular versus burst neuronal activity generally induces less neurotransmitter release (Gonon, 1988), clonidine would be expected to cause a decrease in dopamine turnover in the striatum. Clonidine (Anden and Grabowska, 1976) and another selective α_2-adrenoceptor agonist, medetomidine (MacDonald et al., 1988; Koulu et al., 1993), do indeed cause a decrease in the turnover of dopamine.

Recently it was also suggested that modulation of dopamine release may occur at the level of dopaminergic terminal fields. There is evidence that α_2-adrenoceptor agonists can decrease the release of dopamine from presynaptic terminals in slices of the rat nucleus accumbens (Russell et al., 1993) and rabbit caudate nucleus (Starke et al., 1983; Trendelenburg et al., 1994). Thus, one could propose that there are at least two sites for the interactions between dopamine- and noradrenergic systems. The first is modulation of the activity of dopaminergic cells by means of the somatodendritic adrenoceptors and/or indirect modulations of the dopaminergic cells through some other

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neurotransmitter system(s). The second mechanism would be modulation of the dopamine overflow via the adrenergic receptors on the dopaminergic projecting areas. However, this latter hypothesis requires additional evidence, since only ex vivo studies support it. The present work provided such evidence by means of an in vivo experimental design.

Fast in vivo voltammetry techniques allow one to measure dopamine overflow in 'real-time' and it has been proposed (Gonon, 1988; Stamford et al., 1988, 1991) that when used with electrical stimulation of the dopaminergic ascending pathway, fast in vivo voltammetry provides a way to analyze the effects of drugs on processes which take place at the presynaptic terminals, without artefacts resulting from the effects of these drugs on the firing rate of the dopaminergic neurones. Slow voltammetry, when performed with electrically pretreated carbon fibre electrodes (Gonon et al., 1981; Crespi et al., 1984), allows one to measure extracellular concentrations of dopamine metabolites, thus providing information about the basal level and the overall activity of the DA system.

Therefore, by combining fast and slow in vivo voltammetry methods we aimed to investigate the possible effects of the selective α_2-adrenoceptor agonist, medetomidine, and of the α_2-adrenoceptor antagonist, atipamezole, on the regulation of the dopamine overflow in the mouse caudate nucleus and nucleus accumbens and the effects of these drugs on the basal level of dopamine metabolites–dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the caudate nucleus. Neither of these drugs has any significant affinity for the dopamine receptors (Virtanen et al., 1988, 1989).

2. Methods

2.1. Preparation of animals

All experiments were carried out on male BALB/C mice (25–30 g), bred in the National Animal Centre, Kuopio, Finland. Mice were anaesthetized with chloral hydrate (450 mg/kg i.p.). Anaesthesia was maintained at a level sufficient to prevent corneal reflexes by repeated injections of the anaesthetic at 100 mg/kg every 30–40 min. Rectal temperature was kept at 37 ± 0.5°C by means of a thermostated heating blanket. After removal of a small region of the skull with a dental drill and opening the dura, a working (detector) electrode was placed according to coordinates of the mouse brain atlas (Slotnick and Leonard, 1975) in the caudate nucleus (AP: 0.5 mm, L: 2.0 mm, V: −3.0 mm versus bregma and cortical surface) or nucleus accumbens (AP: 1.2 mm, L: 1.0 mm, V: −4.0 mm). The positions of the working electrodes were verified histologically in some mice after electrolytic marking through the working electrode. This caused irreversible destruction of the electrode. Working electrodes from other mice were calibrated after removal from the brain. A miniature silver/silver chloride reference electrode in a saline bridge was positioned on the contralateral side of the skull. The auxiliary electrode (stainless steel screw) was embedded in the nose bones.

The experiments were carried out with the approval of the local committee on animal welfare.

2.2. Electrical stimulation

A bipolar stimulating electrode with active tips 0.2 mm thick and 0.3 mm long was implanted in the median forebrain bundle (AP: −2.0 mm, L: 1.2 mm, V: −4.9–5.1 mm versus bregma and cortical surface). The electrode was lowered 0.4 mm above the region of interest and stimulation started while the electrode was slowly advanced (1 mm/min). The stimulating electrode was fixed at the depth where the oxidation current at the working electrode was maximal. Electrical stimulation was a continuous constant-current sine wave at 50 Hz, electronically switched on for 4 s periods (high frequency stimulation) or 10 s burst stimulation similar to the normal firing rate of DA neurones (low frequency stimulation). This stimulation consisted of 20 bursts (2 per second). Three sine waves in the burst (with a period of 83.3 ms) were separated by 250 ms between-burst intervals. Thus, 60 pulses at an average frequency of 6 Hz were applied to the stimulation electrode. The root mean square current was normally kept at 80 μA. The stimulations were repeated every 10 min and synchronized with voltammetric measurements to avoid artefacts.

2.3. Electrochemical technique

The dopamine overflow following stimulation of the median forebrain bundle at a high frequency was measured by chronoamperometry with a single untreated carbon fibre, 12 μm in diameter as the working (detector) electrode. The end of the fibre protruding beyond the glass insulation was trimmed to a length of 300 ± 50 μm. The crevices between the carbon fibre and glass insulation at the tip were sealed with epoxy resin. The custom-built three-electrode potentiostat system combined with an IBM computer with digital–analogue and analogue–digital converters for the recording and storage of data and generation of input waveform was used with this carbon fibre electrode. The computer-generated input waveform was a 0.0–0.55 V square pulse, 50 ms width, with current integration from 25 to 48 ms. The catechol oxidation current was monitored at +0.55 V versus Ag/AgCl electrode every 0.25 s (Fig. 1a; Yavich, 1996). Following the experiments, the electrodes were calibrated with solutions of dopamine (1 and 10 μM) and ascorbic acid (200 μM) in 0.1 M phosphate buffered saline (pH = 7.4). The dopamine overflow following stimulation of the median forebrain bundle at low frequency was measured by constant current amper-

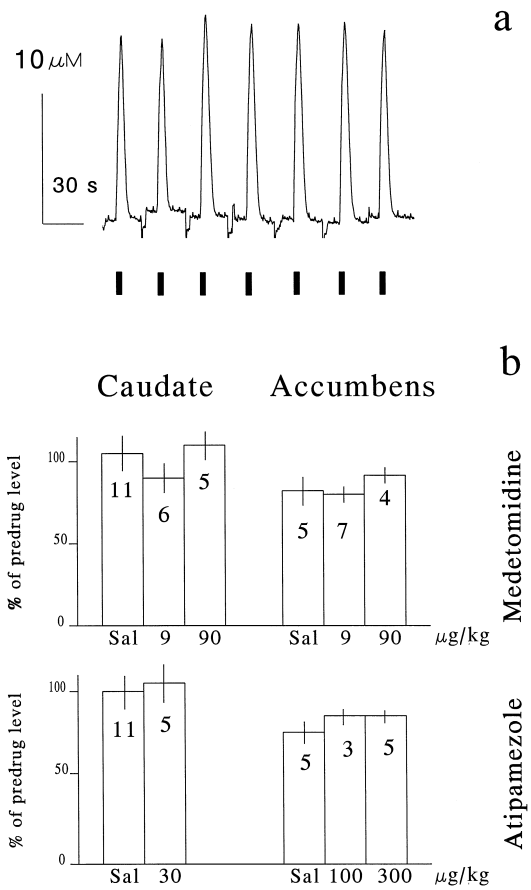

Fig. 1. Example of chronoamperometric recordings obtained with carbon fibre electrodes in the mouse caudate nucleus (a) and the effects of medetomidine and atipamezole on the DA overflow in the mouse caudate and nucleus accumbens induced by high (50 Hz) frequency stimulation of the median forebrain bundle (b). (a) Dopamine overflow following 4 s 50 Hz electrical stimulation (bars) of the median forebrain bundle at 10 min intervals. Chronoamperometric difference current obtained with untreated carbon fibre electrode every 0.25 s. (b) Each column represents maximal DA overflow as a percentage of the predrug level (mean ± S.E.M.) 30 min after drug/saline administration. Number of mice in each experiment is shown in the columns. Note: there were no significant differences in these experiments.

ometry with electrically treated carbon fibre electrodes. Electrodes were constructed as described above and pretreated before the experiment with triangular pulses 1.0–2.4 V at 6 Hz for 30 s in phosphate buffered saline (Ghasemzadeh et al., 1993). This treatment significantly increased the sensitivity of the electrodes to catechols, making possible recording of a nanomolar concentration of dopamine at the low frequency stimulation. The catechol oxidation current was monitored at +0.25 V versus Ag/AgCl electrode. Electrodes were calibrated as described above, but with a lower concentration of dopamine (0.1 and 1 μM). The basal levels of DOPAC and HVA were measured using differential pulse voltammetry with a carbon fibre electrode electrically treated before implantation for the registration of 4 peaks (Fig. 4a; Crespi et al., 1984): ascorbic acid at −50 mV (peak 1), DOPAC at

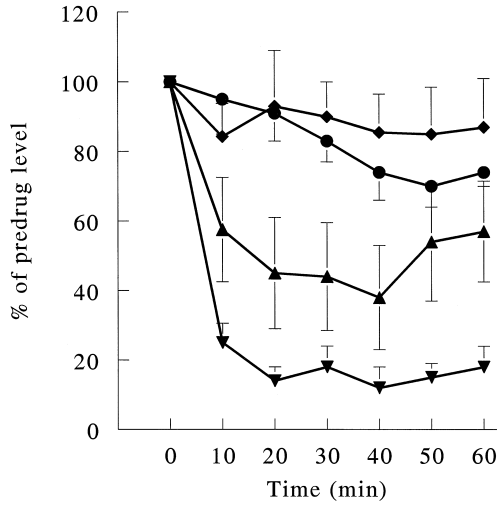

Fig. 2. The effects of medetomidine on the DA overflow induced by low (6 Hz average) frequency stimulation of the median forebrain bundle in the mouse nucleus accumbens. Each data point represents the maximal DA overflow as a percentage of the predrug level (mean ± S.E.M.) and is marked as: (♦) saline, $n = 7$, (●) medetomidine 5 μg/kg, $n = 6$, (▲) medetomidine 20 μg/kg, $n = 6$, (▼) medetomidine 100 μg/kg, $n = 6$.

+80 mv (peak 2), 5-hydroxyindoleacetic acid (5-HIAA) and uric acid at +250 mV (peak 3) and HVA at +350 mV (peak 4). The quality of each electrode after pretreatment was established in phosphate buffered saline contain-

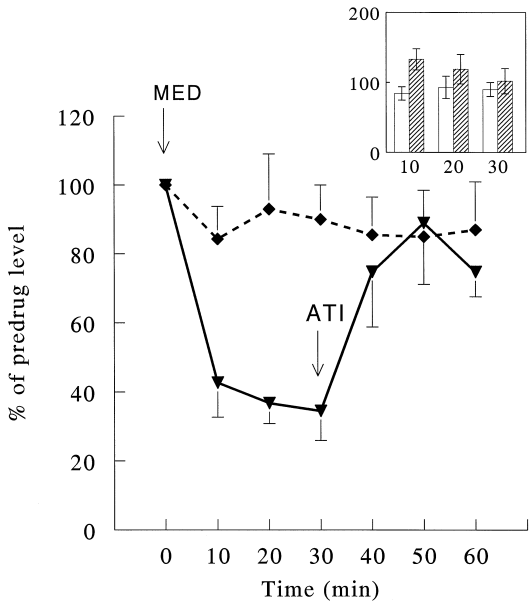

Fig. 3. Time-course of the effects of medetomidine on the evoked DA overflow in the mouse nucleus accumbens and antagonism of these effects by atipamezole treatment. Each data point represents the maximal DA overflow as a percentage of the predrug level (mean ± S.E.M.). Control group (♦, saline, $n = 7$) is the same as in Fig. 2. Drugs (▼), medetomidine 100 μg/kg, $n = 6$ (MED) and atipamezole 300 μg/kg (ATI) were injected immediately after the measurement at the time points marked by arrows. Insert: the effects of atipamezole alone (300 μg/kg, $n = 6$) on the DA overflow in nucleus accumbens 10, 20 and 30 min after administration.

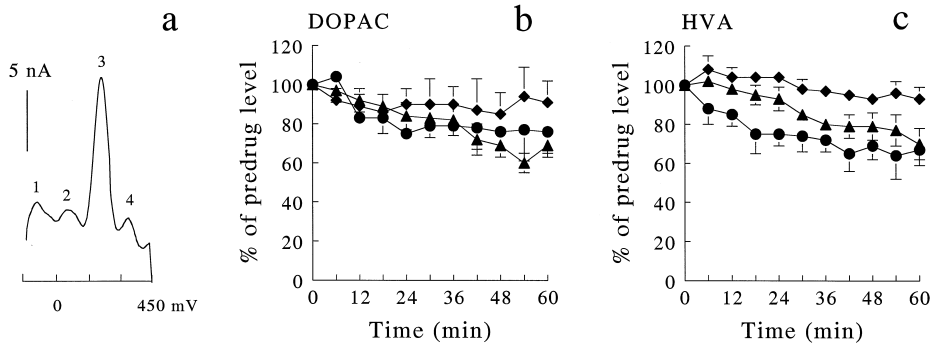

Fig. 4. Example of differential pulse voltammetry recordings obtained with carbon fibre electrodes in the mouse caudate nucleus (a) and time course of the effects of medetomidine and atipamezole on the concentrations of DOPAC (b) and HVA (c) in mouse caudate nucleus. (a) Differential pulse voltammetry with electrically pretreated carbon fibre electrode gives 4 peaks: (1) ascorbic acid, (2) DOPAC, (3) 5-HIAA and uric acid (about 30%), (4) HVA. Interval between scans was 6 min. (b, c) Each data point represents the height of peak 2 (DOPAC, panel b) or peak 4 (HVA, panel c) as a percentage of the predrug level (mean ± S.E.M.). Medetomidine (●, 90 μg/kg $n = 11$), atipamezole (▲, 300 μg/kg, $n = 5$) or saline (♦, $n = 11$) were injected immediately after the recording at '0' time point.

ing 200 μM of ascorbic acid, 20 μM of DOPAC, 2.5 μM of 5-HIAA and 5 μM of HVA.

2.4. Data presentation and statistics

All experiments began at least one hour after implantation of the detector electrode. In the experiments involving the recording of the evoked dopamine overflow, drug or saline was injected (5 ml/kg, s.c.) immediately after the third stimulation of the median forebrain bundle that gave a stable dopamine release. The time courses of the effects of medetomidine were established for at least 1 h after injection, using 10 min intervals between stimulation. The time course of the effects of atipamezole alone was studied for only 30 min providing a control for the experiments involving combination of medetomidine and atipamezole. In these experiments, atipamezole was injected 30 min after medetomidine (immediately after the stimulation of the median forebrain bundle) and the effects were monitored for a further 30 min. The action of the drugs on the peak amplitude of the evoked dopamine overflow was expressed as a percentage of the peak effect of the last stimulation recorded before drug injection. In the experiments involving the recording of the basal level of DOPAC and HVA, drugs or saline were injected immediately after the recording. Time courses of the effects of the drugs on the amplitudes of peak 2 (DOPAC) and peak 4 (HVA) were measured for 1 h after treatments at intervals of 6 min and expressed as percentages of the last recording before administration of drug. Medetomidine and atipamezole were gifts from Orion Corporation (Finland). All other chemicals and drugs were purchased from Sigma (USA).

Statistical analyses of the effects of drugs were performed using a multivariate analysis of variance (MANOVA) for repeated measures with drug treatments as between-subject factor and repeated electrical stimulations (the first experiment) or repeated measures of metabolite concentrations (the second experiment) as the within-subject factor. The stability of dopamine overflow after repeated stimulations or the amplitudes of peaks 2 (DOPAC) and 4 (HVA) after repeated measurements was analyzed using a multivariate test of significance (Pillais statistics) or else an averaged test. Statistical analysis of the effects of combination of medetomodine and atipamezole involved two levels between-subject factor (saline- and drug-treated groups) and two within-subject factors (treatment — two levels and repeated electrical stimulation — three levels). The individual differences between controls and the overall effects of drugs were analyzed using univariate *t*-tests for between-subject effects. The effects of drugs at different time after treatments were analyzed using the MANOVA univariate *F*-tests with Bonferroni correction. All data are presented as arithmetical means ± standard error (S.E.M.).

3. Results

High-frequency (50 Hz) stimulation of the median forebrain bundle for 4 s induced a rapid dopamine release (10.6 ± 1.4 μM, $n = 11$) in the caudate nucleus which was stable in amplitude at 10 min stimulation intervals ($F(4) = 1.28$, $P = 0.31$). The concentration of the released dopamine in the nucleus accumbens was 14.9 ± 2.1 μM ($n = 8$). Repeated stimulation of the median forebrain bundle induced some decrease in dopamine release ($F(4) = 8.3$, $P = 0.000$) on the 5th and 6th stimulation (after saline injection) in nucleus accumbens. The dopamine overflow in the caudate and accumbens nuclei showed no significant differences ($n = 19$, 2-tailed Mann–Whitney *U* test, $P = 0.15$). Fig. 1a shows an example of a chronoamperometric recording following stimulation of the median forebrain bundle.

Neither medetomidine at 9 and 90 μg/kg nor atipamezole at 30, 100 and 300 μg/kg induced any changes in the

evoked dopamine overflow in the caudate nucleus or nucleus accumbens following high frequency stimulation of the median forebrain bundle. Thus, the results are shown only for one time point (30 min after administration of the drugs in comparison to the effects of saline at the same time point, Fig. 1b).

Subsequent experiments utilized low (6 Hz average) frequency stimulation of the median forebrain bundle. In these experiments the working electrodes were positioned in the nucleus accumbens. Post-calibration of the electrodes was performed for the group treated with saline. All other working electrodes were used for electrolytic marking of their positions within the nucleus accumbens. Low frequency stimulation of the median forebrain bundle for 10 s induced peak dopamine release at a level of 0.40 ± 0.04 μM ($n = 8$). The responses were stable in amplitude following repeated stimulation at 10 min intervals ($F(6) = 0.41$, $P = 0.87$). In contrast to the previous experiments, now medetomidine clearly decreased the evoked dopamine overflow. Some non-significant decrease was detected even at a dose as low as 5 μg/kg (Fig. 2). At higher doses, medetomodine, dose dependently and significantly decreased dopamine overflow (between-subjects effects: $F(3) = 12.47$, $P = 0.001$; individual estimates: 20 μg/kg $- t = 3.63$, $P = 0.002$; 100 μg/kg $- t = 2.72$, $P = 0.01$). This effect reached 85% at a dose of 100 μg/kg. Atipamezole at a dose of 300 μg/kg antagonized the effects of medetomidine at 100 μg/kg when injected 30 min after medetomidine, restoring dopamine overflow up to the control levels (groups by treatments interactions: $F(1) = 20.77$, $P = 0.001$, Fig. 3). Atipamezole also protected mice against the action of medetomidine when it was injected 5–10 min before the agonist (data not shown). The effects of atipamezole alone on the dopamine overflow evoked at low-frequency stimulation were not significant ($F(1) = 1.69$, $P = 0.22$, Fig. 3, insert).

The effects of medetomidine at 90 μg/kg and atipamezole at 300 μg/kg on the basal level of DOPAC and HVA in mouse caudate were investigated in subsequent experiments, using differential pulse voltammetry (Fig. 4a). Medetomidine decreased the concentration of HVA in the caudate nucleus in comparison to control experiments ($F(1,10) = 3.24$, $P = 0.001$). The first significant ($P < 0.05$) effect of the drug was obtained 12 min after treatment (Fig. 4c). The maximal decrease in metabolite concentration was seen at the end of the test period 40–60 min after injection of medetomidine and did not exceed 35%. The slight decrease in DOPAC concentration was not significant. Atipamezole had no effects on the level of dopamine metabolites in the mouse caudate (Fig. 4b, c).

4. Discussion

We found that the α_2-adrenoceptor agonist, medetomidine, at a dose of 90 μg/kg induced a 35% decrease of the concentration of HVA in the mouse caudate nucleus. This may indicate a reduction of dopamine turnover in dopaminergic areas of the mouse brain. Similar results have been obtained in rats, using other experimental approaches. Thus, α_2-adrenoceptor agonists which lack dopaminergic activity, such as clonidine (Anden and Grabowska, 1976), also reduced dopamine turnover. Medetomidine reduced the concentration of HVA and decreased the rate of dihydroxyphenylalanine (DOPA) accumulation in the caudate but not in the nucleus accumbens and substantia nigra in rats, and this effect could be antagonized by the specific α_2-adrenoceptor blocker, idazoxan (MacDonald et al., 1988; Koulu et al., 1993). In rats, clonidine was very effective against the effects of haloperidol on dopamine turnover, though its own activity was weak (Waldmeier et al., 1982). On the other hand, α_2-adrenoceptor antagonists which do not possess intrinsic activity at dopamine receptors have little or no effect on dopamine metabolism in the striatum (Waldmeier et al., 1982), in whole rat brain (Scheinin et al., 1988) and on stimulated dopamine release in slices from rat nucleus accumbens (Nurse et al., 1985; Russell et al., 1987; Russell et al., 1993). Thus, our results confirm previous findings that atipamezole is ineffective to modulate dopaminergic activity in striatum.

The decrease in dopamine turnover in striatum after α_2-adrenoceptor agonist treatment may be due to the modulation of the firing rate of mesolimbic and mesostriatal dopaminergic neurones directly or via the inhibition of adrenergic tone from the locus coeruleus (Herve et al., 1982; Grenhoff and Svensson, 1989; Lategan et al., 1990, 1992). However, some recent findings permit us to suggest another site of action of α_2-adrenoceptor agonists. Since medetomidine reduced dopamine turnover in the caudate nucleus but did not cause any change in dopamine turnover in a cell body area (Koulu et al., 1993) it was proposed that the changes in metabolite level are mediated via the α_2-adrenoceptors located in the caudate nucleus. The dense distribution of the α_2-adrenoceptors (Boyajian et al., 1987; Biegon et al., 1992; Hudson et al., 1992; Rosin et al., 1996) in rat caudate-putamen, nucleus accumbens and olfactory tubercle i.e. dopaminergic projection areas is consistent with this suggestion. A similar distribution was recently shown in mice (Link et al., 1995). Further convincing data were obtained from investigations of adrenergic modulation of dopamine release in slices. The α_2-adrenoceptor agonists, clonidine and UK14304, are able to inhibit dopamine release from rat nucleus accumbens slices in response to potassium and low-frequency electrical stimulation (5 Hz) and this effect could be antagonized by idazoxan. However, this was not seen in rat caudate slices (Nurse et al., 1985; Russell et al., 1987; Russell et al., 1993). Inhibition of dopamine release has been obtained with UK 14304 in rabbit caudate slices following brief high-frequency stimulation (6 pulses/100 Hz), which induced about 85 nM peak dopamine release (Trendelenburg et al., 1994).

In our in vivo experiments, medetomidine injected systemically at low concentrations dose dependently decreased the electrochemical response evoked from a detector electrode. Several lines of evidence support the view that stimulation of the median forebrain bundle induces dopamine release from striatum, and in vivo voltammetry is a unique technique to measure stimulated DA overflow without causing unwanted interference by ascorbic acid and dopamine metabolites (Kuhr et al., 1984; Millar et al., 1985; Gonon, 1988; Suaud-Chagny et al., 1989, 1992). Mice are not commonly used in in vivo voltammetric experiments. However, we have previously shown that dopamine overflow is truly recorded following stimulation of the median forebrain bundle in mice (Yavich, 1996). Thus, medetomidine decreased the stimulated dopamine release in the in vivo experiments in anaesthetized mice. This seems specific for α_2-adrenoceptor agonists, since atipamezole restored the dopamine overflow but itself had no clear effects on dopamine release. Peripheral injections of drugs may change the firing rate of dopaminergic neurones and lead to an increase or decrease in dopamine release. However, with stimulated release, these kinds of effects are not detected, since the stimulus applied externally overwhelms any neuronal tone. In other words, if medetomidine or atipamezole affected dopamine release only at the level of the cell body or dendrite, their effects would not be detected in these experiments. Our results permit us to conclude that the activation of α_2-adrenoceptors at the level of the striatum is the reason for the observed effects of medetomidine. It is worth noting that the decrease of evoked dopamine overflow after medetomidine treatment was seen only with a low frequency of stimulation (0.4 μM peak dopamine release), similar to the normal burst activity of dopaminergic neurones (Grace and Bunney, 1984). Dopamine overflow following high-frequency stimulation (10–15 μM peak dopamine release) was not affected. This may be due to masking of the effects of α_2-adrenoceptor agonists by excessive activation of the autoinhibitory presynaptic dopamine receptors.

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