ELSEVIER

European Journal of Pharmacology 273 (1995) 247–257

ejp

Acute dopaminergic influence on plasma adrenaline levels in the rat

Mirjana Kujacic *, Lars O. Hansson, Arvid Carlsson

Department of Pharmacology, University of Göteborg, Medicinaregatan 7, S-413 90 Göteborg, Sweden

Received 9 June 1994; revised 10 November 1994; accepted 11 November 1994

Abstract

This study was aimed at in vivo characterisation of the possible role of dopamine receptors in the modulation of adrenaline release from the adrenal medulla in rats. Quinpirole (0.3, 1 and 3 mg/kg s.c., 30 min), an agonist at dopamine D_2-like receptors, induced a statistically significant increase not only in adrenal dopamine but also in plasma and heart adrenaline levels. The effects of the lowest dose of quinpirole were blocked by domperidone (5 mg/kg s.c., 150 min). Implantation of catheters followed by blood sampling appeared to be a stressful procedure, inducing itself an elevation of adrenal dopamine and of heart adrenaline by 100 and 250%, respectively. To explore the possibility of determining the plasma levels of adrenaline without blood sampling, regression modelling was performed by means of partial least squares regression (PLS) using treatment and levels of heart adrenaline and adrenal dopamine as predictor variables. The selected variables were found to be good predictors of plasma adrenaline levels. Accordingly, the increase in adrenal dopamine and heart adrenaline levels following administration of the dopamine autoreceptor agonist, talipexole, and the classical non-selective dopamine receptor agonist, apomorphine, were interpreted as indicators of the increased adrenomedullary adrenaline release. Neither of the dopamine D_2 receptor antagonists used, i.e. domperidone, supposed to have only peripheral effects, nor raclopride, had significant effects on adrenal dopamine and heart adrenaline. Our results support the presence of peripherally located dopamine D_2-like receptors, capable of acutely stimulating not only the synthesis of catecholamines, but also the release of adrenaline from adrenals in the conscious rat.

Keywords: Dopamine receptor; Adrenaline; Heart; Plasma; Dopamine; Adrenal medulla; (Partial least squares regression)

1. Introduction

Dopamine D_2 receptors have been identified in vitro on bovine adrenomedullary chromaffin cells in receptor binding studies (Gonzalez et al., 1986; Lyon et al., 1987; Quik et al., 1987; Bigornia et al., 1990) and by in situ hybridisation histochemistry in the rat (Schalling et al., 1990). Our previous results indicate that the stimulation of dopamine D_2-like receptors, probably peripherally located, increases catecholamine synthesis in the rat adrenal glands (Kujacic et al., 1990,1991; Kujacic and Carlsson, 1993). The question remains of whether the dopamine receptors also play a role in the modulation of catecholamine release from adrenal medulla.

Recently, several laboratories have evaluated the regulation of catecholamine release from adrenal tissue by dopamine receptors. The results are, however, far from conclusive. The results obtained in vitro with stimulated perfused adrenal glands (Collett and Story, 1982 [rabbit]; Artalejo et al., 1985; Montiel et al., 1986 [cat]; Gonzalez et al., 1986 [bovine]), or with isolated bovine chromaffin cells in culture (Bigornia et al., 1988,1990; Sontag et al., 1990) suggest the presence of dopamine receptors on chromaffin cells capable of inhibiting catecholamine release from the adrenal medulla. However, the concentrations of dopaminergic agents used in these experiments in vitro were much higher than those presumably prevailing under in vivo conditions. A further complication was the report of dopamine D_1 receptor-mediated facilitation of Ca^{2+} currents in bovine chromaffin cells (Artalejo et al., 1990). On the other hand, Huettl et al. (1991), in experiments on bovine chromaffin cell culture, ruled out a role of dopamine receptors in the modulation of catecholamine release.

Results obtained in vivo mostly evidence an inhibitory influence of peripheral dopamine receptors on

* Corresponding author. Tel. (46) 031 773 34 00, fax (46) 031 81 17 95.

SSDI 0014-2999(94)00699-7

catecholamine release from adrenals. In anaesthetised and vagotomised dogs with electrically stimulated splanchnic nerves, the dopamine D_2-like receptor agonist, quinpirole, had an inhibitory effect, while the dopamine D_2-like receptor antagonist, domperidone, had a stimulatory effect on noradrenaline and adrenaline release (Foucart et al., 1988). Stimulation of noradrenaline and adrenaline release was induced in humans by domperidone during exercise (Mercuro et al., 1988; Mannelli et al., 1988) or after glucagon stimulation (Mannelli et al., 1990). However, experiments performed on conscious rats (Nagahama et al., 1986; Regunathan et al., 1989) indicate the opposite effects, i.e. that dopamine receptor agonists stimulate adrenaline release from the adrenals, suggesting the possibility of species differences.

The present experiments were performed to investigate the effects of dopamine D_2 receptor ligands on the release of adrenaline from the adrenal medulla in rats. It was found that the blood sampling procedure itself caused an elevation of plasma adrenaline levels. To control for this factor plasma adrenaline levels were calculated, based on a regression model using adrenal dopamine and heart adrenaline levels. Adrenal dopamine levels have previously been shown to be a reliable index of adrenal catecholamine synthesis (Kujacic and Carlsson, 1993 and references therein).

2. Materials and methods

2.1. Animals

All studies were performed on male rats of the Sprague-Dawley strain (ALAB, Stockholm, Sweden), weighing 200–300 g. The rats were housed in groups of five per cage under standard environmental conditions for at least a week from their arrival until being used in the experiments. Laboratory chow (R3, Ewos, Södertälje, Sweden) and tap water were freely available. The experimental animals were handled as carefully as possible in order to minimise environmental stress.

2.2. Drugs

The following drugs were used: quinpirole hydrochloride (Ly 171555) (Eli Lilly & Co., Indianapolis, IN, USA), talipexole dihydrochloride (B-HT 920) (Boehringer Ingelheim, Ingelheim am Rhein, Germany), apomorphine hydrochloride (Sandoz, Basle, Switzerland), raclopride tartrate (Astra Läkemedel, Södertälje, Sweden), domperidone (Janssen Pharmaceutica, Beerse, Belgium), SCH 23390 (7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol) (Schering Corp., Bloomfield, NJ, USA) and SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine) (Smith, Kline and French, Philadelphia, NJ, USA).

Quinpirole hydrochloride, talipexole dihydrochloride, apomorphine hydrochloride and raclopride tartrate were dissolved in physiological saline (0.9% NaCl); SKF 38393 was dissolved in a small amount of glacial acetic acid and diluted with saline; SCH 23390 and domperidone were dissolved in minimal quantities of glacial acetic acid and diluted with 5.5% warm glucose solution. Solutions were adjusted to pH 5–6 with 0.1 M sodium hydroxide. Control groups were injected with physiological saline. All compounds were injected subcutaneously into the neck in a volume of 5 ml/kg body weight.

2.3. Dissection, tissue extraction and analyses of catecholamines

After various periods of drug administration the rats were decapitated. The adrenal glands, hearts and brains were rapidly taken out and dissected. The adrenal glands and hearts were frozen immediately on dry ice, while the brains were placed on chilled Petri dishes and divided into two parts by means of a frontal section at the median eminence. Only the dopamine-rich anterior part of the brain was saved and frozen on dry ice. Tissue parts were weighed and stored at −70°C until analysis.

Frozen tissue was homogenised with an Ultra-Turrax homogeniser in 3 ml of 0.1 M perchloric acid, 50 μl of 10% sodium EDTA and 50 μl of 5% glutathione (forebrain, adrenals), or 9 ml of 0.1 M perchloric acid, 150 μl of 10% sodium EDTA and 150 μl of 5% glutathione (heart). After centrifugation (10 000 × *g*, 0°C, 10 min) followed by filtration, 1 ml of the supernatant was taken for analysis of noradrenaline, adrenaline, 3,4-dihydroxyphenylacetic acid (DOPAC) and dopamine. The supernatant from the adrenal glands, was diluted 10-fold.

Tissue levels of noradrenaline, adrenaline, DOPAC and dopamine were determined according to standard techniques by means of high performance liquid chromatography with electrochemical detection (HPLC-EC) (for further methodological details, see Kujacic et al., 1990).

2.4. Blood sampling and catecholamine assay

One day before the experiment the rats were anaesthetised with ketamine (Ketalar, Parke-Davis, 50 mg/ml) + xylazine (Rompun, Bayer, 20 mg/ml); the mixture contained 2 volumes Ketalar and 1 volume Rompun, and was injected i.p., 2 ml/kg. Under anaesthesia, catheters (PE_{50} polyethylene tubing, i.d. 0.58 mm) were implanted in the left carotid artery and the right jugular vein and exteriorised at the back of the

neck – from the right vein to the left and left artery to the right, with free endings of 1–2 cm. The catheters were filled with heparin solution (1000 IU) in order to prevent blood coagulation and the catheter endings were obstructed with a needle to prevent bleeding. After surgery the rats were housed individually and had free access to their regular chow and water.

Just before killing, the blood samples (2.0 ml) were slowly collected (within 2 min) from the carotid artery into a plastic syringe and then immediately transferred into cold heparinised tubes (50 IU heparin) placed on ice and containing 40 μl 0.4 M (0.125 g/ml H_2O) glutathione solution. The blood drawn from the artery was replaced with the same volume of 0.9% saline with 50 IU heparin injected into the jugular vein. During the blood collection the rats were allowed to move freely in their individual cages.

The blood was centrifuged promptly for 10 min. The plasma was removed, frozen and stored at −70°C until analysis. For each simultaneous analysis of noradrenaline, adrenaline and dopamine 1 ml plasma was used (if only a smaller volume could be collected, the necessary calculations were done).

The plasma catecholamines were purified and concentrated by extraction with aluminium oxide. The extraction procedure was performed as follows: 50 μl glutathione (1%), 20 mg acid-washed aluminium oxide, 2 ng α-methyl dopamine (in 50 μl solution containing 0.1 M perchloric acid and 0.015% glutathione, e.g. 40 ng/ml) as the internal standard and, under vigorous stirring, 0.5 ml of 3 M Tris buffer (pH 8.6) with 1% EDTA were added to 1 ml of plasma, or external standard. The tubes were subsequently vigorously shaken for 15 min, following which the liquid was removed and the aluminium oxide was washed 4 times with water. After the last washing the supernatant was removed as completely as possible. After this the catecholamines were eluted with 75 μl 0.2 M perchloric acid with 0.015% glutathione and stored at −20°C.

External standard samples were prepared in the following manner: standard solutions of noradrenaline, adrenaline and dopamine (50 μg/ml) were mixed and diluted to 1 ng/ml with 0.1 M perchloric acid with 0.015% glutathione. As already mentioned, the extraction procedure was performed in the same manner as with plasma samples.

After desorption of the catecholamines from the aluminium oxide, 20 μl of supernatant or external standard solution was injected into the HPLC system, as previously described (Kujacic et al., 1990). The only difference was in the choice of stationary phase. A cation exchange column (0.46 × 15 cm) packed with Nucleosil 5 SA (Macherey-Nagel, Düren, Germany) was used instead of the reversed phase column (Nucleosil, RP-18, 5 μm) previously used.

The mobile phase contained: 0.09 M citric acid · H_2O, 0.19 M sodium hydroxide, 10% v/v of methanol and sodium EDTA (0.215 mM). The flow rate was 1.2 ml/min.

2.5. Statistics

Fisher's PLSD (protected least significant difference) test preceded by one-way analysis of variance (ANOVA) was applied (Milliken and Johnson, 1984). Probability levels of less than 0.05 were regarded as significant.

For the multivariate calculations the Sirius, ver. 3.0, Sirius for Windows, ver. 1.1 program packages (Pattern Recognition Systems, Bergen, Norway) and a demo version of the Simca-S for Windows, ver. 5.01 program package (Umetri, Umeå, Sweden) were used.

3. Results

3.1. Effects of quinpirole and domperidone on adrenal dopamine, and plasma and heart adrenaline levels when blood sampling was performed

Quinpirole, a dopamine D_2-like receptor agonist, in all doses tested (0.3, 1 and 3 mg/kg, 30 min) induced a marked increase in plasma adrenaline, by 200–600% (Fig. 1) compared to the saline-treated controls. At the same time, quinpirole at all doses induced a statistically significant increase in adrenal dopamine and heart adrenaline (Fig. 1).

Domperidone, a dopamine D_2 receptor antagonist, not supposed to readily cross the blood-brain barrier, injected in a dose of 5 mg/kg s.c. 150 min before decapitation, was not able to block the effects of 3 mg/kg quinpirole (30 min) either on adrenal dopamine, or on plasma adrenaline and heart adrenaline (Fig. 2a). On the other hand, when a 10-fold lower dose of quinpirole was used (0.3 mg/kg) its effects on adrenal dopamine, plasma adrenaline and heart adrenaline were blocked by domperidone (Fig. 2b).

Domperidone did not bring about significant changes in forebrain DOPAC (data not shown), a finding which supported its assumed exclusively peripheral activity.

3.2. Stress-inducing effect of the blood sampling procedure

To assess the effect of the implantation of arterial and venous catheters, followed by the blood sampling procedure on forebrain DOPAC, adrenal dopamine and/or heart adrenaline, the following groups were compared: group A, which was kept under the same experimental conditions as the control groups in our experiments without blood sampling (not operated, but injected with physiological saline), group B (operation

plus injection of physiological saline, no blood sampling) and group C, which was kept under the same conditions as the control group in the experiments with blood sampling (operation plus blood sampling plus injection of physiological saline). No statistically significant changes in forebrain DOPAC were observed, but the adrenal dopamine and heart adrenaline were increased in group C by about 100% and 250%, respectively, compared to those in group A (Fig. 3). The operation procedure seemed to induce most of the increase in adrenal dopamine and heart adrenaline, while the blood sampling induced only a further increase in heart adrenaline, but did not cause any changes in adrenal dopamine (Fig. 3).

3.3. Prediction of plasma adrenaline levels

As shown above, the blood sampling technique used here apparently greatly stresses the test animals. Therefore, we wanted to investigate the possibility of estimating indirectly the plasma levels of adrenaline without any sampling of blood. To accomplish this different regression modelling techniques were tried, such as multiple regression or partial least squares regression (PLS) (Wold, 1975; Clark and Cramer, 1993). Here PLS was used to obtain predictive models.

The series of experiments with saline controls, the dopamine D_2-like receptor agonist, quinpirole, the dopamine D_2 receptor antagonist, domperidone, and combinations of these drugs where blood samples were collected from the test animals ($n = 47$, see Figs. 1 and 2) was used for the calibration model. A regression model was calculated, using adrenal dopamine, heart adrenaline and administered drugs/dose(s) as the independent variables. The information about the administered drugs was given an indicator variable (1 = not administered, or 2 = administered) to denote the seven different possibilities for treatment (T_{1-7}). The logarithm of the plasma levels of adrenaline was used as the dependent variable. All concentration values included were expressed as percent of controls. The variables were scaled to unit variance and zero mean (auto scaling, see Wold et al., 1984; Dunn and Wold, 1990). The resulting statistically significant (cross-validation, see Stone, 1974; Wold, 1978) two-component model accounted for 44.8% of the variance in the independent variable block and for 93.4% of the variance in the dependent variable (log[plasma adrenaline]). A conservative estimation of the validity of the model by means of the cross-validated r^2 (Clark and Cramer, 1993) ($r^2_{cv} = 0.909$, $n = 47$, 9 independent variables) indicated a significance well beyond the 99.9% level. The r^2_{cv} is a measure that is quite analogous to the classical r^2, but it also measures the predictive properties of the model instead of only its data-fitting ability. The resulting regression equation is given in Fig. 4a together with a graph representing the relation between the calculated and the experimentally found arterial plasma levels of adrenaline.

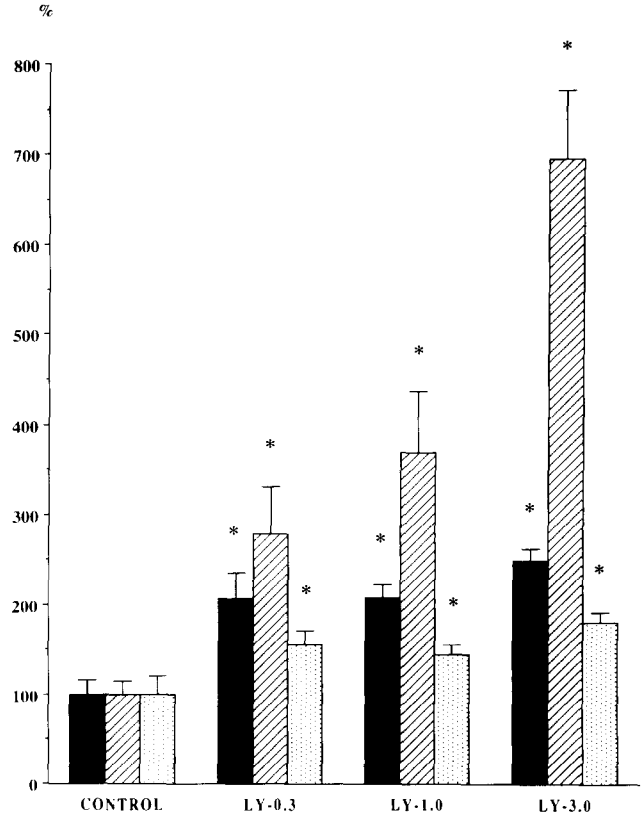

Fig. 1. Changes in adrenal dopamine, and arterial plasma and heart adrenaline in rats caused by quinpirole. $n = 4–5$. Quinpirole (LY) in doses of 0.3–3 mg/kg was administered s.c., 30 min before decapitation. Data were obtained in two separate experiments: in the first one quinpirole was administered in doses of 0.3 and 1 mg/kg (i) and in the second one in a dose of 3 mg/kg (ii). Data are shown as the means ± S.E. expressed as the percent of the value for the saline-treated controls (i: 317 ± 28 ng/pair adrenal dopamine, 0.43 ± 0.03 ng/ml plasma adrenaline, 52 ± 3 ng/g heart adrenaline; ii: 351 ± 56 ng/pair adrenal dopamine, 0.41 ± 0.06 ng/ml plasma adrenaline, 70 ± 15 ng/g heart adrenaline). * $P < 0.05$ (vs. corresponding control). Dark columns: adrenal dopamine; hatched columns: plasma adrenaline; stippled columns: heart adrenaline.

To further evaluate the validity of the relation found, 11 observations were excluded and the model was recalculated. This resulted in a two component model ($r^2_{cv} = 0.92$) by which the plasma adrenaline levels of the excluded observations could be predicted with very high accuracy (see Fig. 4b). The changes in the regression coefficients compared to the former equation were only minute (not shown).

The PLS regression model was then further utilised to estimate the plasma levels of adrenaline in animals where no sampling of blood was done. Here, the logarithm of the absolute levels of adrenal dopamine and heart adrenaline was used instead of percent of controls (Fig. 4c). A calculation similar to that above

Table 1
Plasma levels of adrenaline calculated from the model shown in Fig. 4c, with treatment data included, in the rats in which blood sampling was not performed.

Treatment	Measured value			Calculated value		
	ng/ml	%	n	ng/ml	%	n
Saline	0.48 ± 0.04	100 ± 8	10	0.31 ± 0.01	100 ± 3	4
Quinpirole (0.3 mg/kg)	1.78 ± 0.27	371 ± 56	7	0.77 ± 0.03	248 ± 10	8
Quinpirole (3.0 mg/kg)	2.82 ± 0.31	588 ± 65	5	1.13 ± 0.06	365 ± 19	4

Value measured refers to the plasma adrenaline levels actually determined following blood sampling. Data are shown as the means ± S.E.

yielded a significant (cross-validation $r^2_{cv} = 0.852$) two-component model where 42.2% of the variance of the independent variable block and 89.2% of the dependent variable (log[plasma adrenaline]) was accounted for. This model was then used to calculate the levels of plasma adrenaline in saline-treated rats and in rats treated with quinpirole at 0.3 mg/kg and 3 mg/kg. A summary of these results is shown in Table 1. The values measured for arterial plasma adrenaline were statistically significantly greater ($P < 0.05$) than the values calculated for all three corresponding groups. Furthermore, the quinpirole-induced increase in adrenaline release appears to be potentiated by the stress (induced by blood sampling), as the values measured in saline-treated rats were higher by 55% than the calculated values, while in quinpirole-treated groups this difference exceeded 130–150%.

3.4. Effects of the dopamine receptor agonists, quinpirole, talipexole and apomorphine, on adrenal dopamine and heart adrenaline levels when blood sampling was not performed

Quinpirole, injected in doses between 0.03–3 mg/kg, induced a statistically significant increase in adrenal

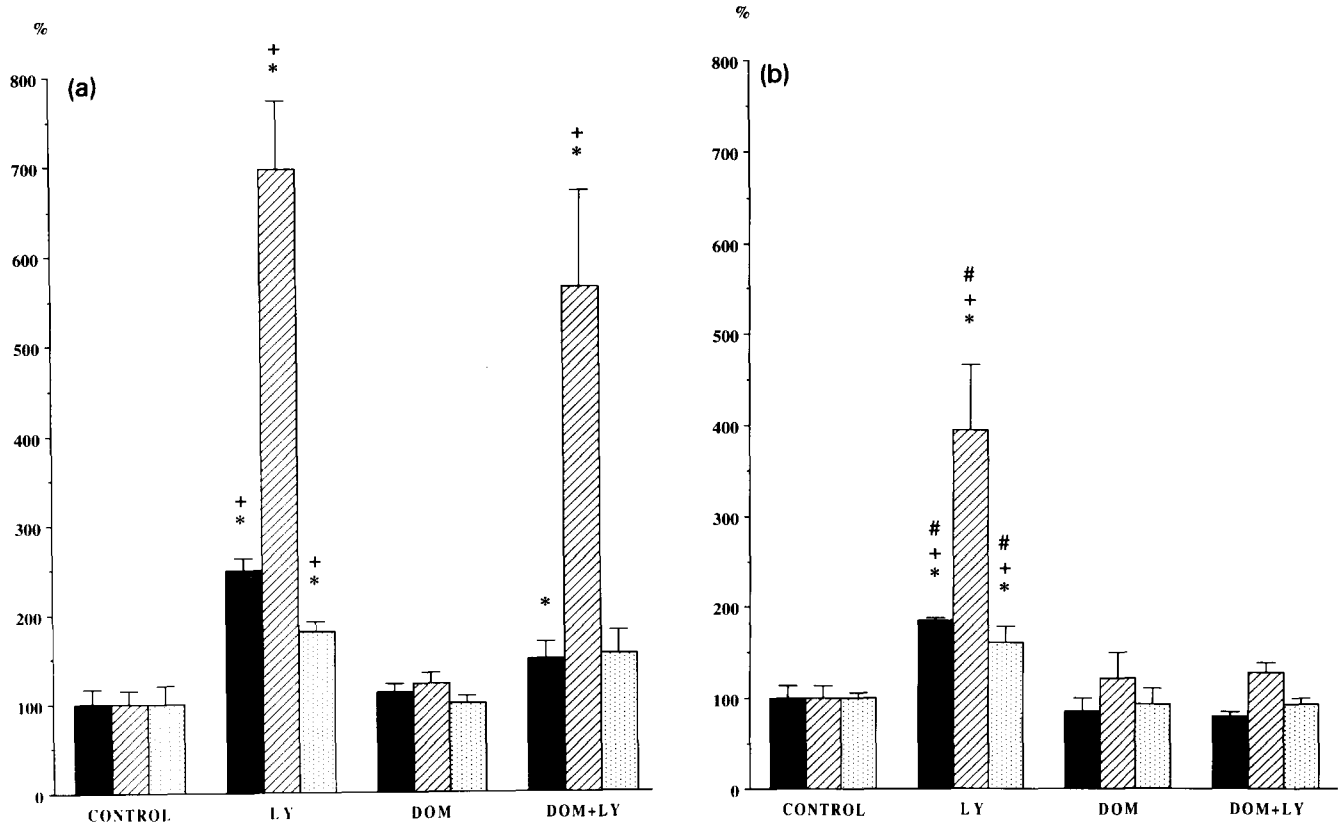

Fig. 2. Effects of domperidone on quinpirole-induced changes in adrenal dopamine, and arterial plasma and heart adrenaline in rats. Data were obtained from two separate experiments (a, b). $n = 4–5$ in each group. Quinpirole (LY) 3 mg/kg s.c. (a), or 0.3 mg/kg (b) was administered 30 min and domperidone (DOM) 5 mg/kg s.c., 150 min before decapitation. The data are shown as the means ± S.E. expressed as percent of the value for the saline-treated controls (a: 351 ± 55 ng/pair adrenal dopamine, 0.41 ± 0.06 ng/ml plasma adrenaline, 70 ± 15 ng/g heart adrenaline; b: 323 ± 45 ng/pair adrenal dopamine, 0.54 ± 0.08 ng/ml plasma adrenaline, 80 ± 4 ng/g heart adrenaline). * $P < 0.05$ (vs. corresponding control), $^{+}$ $P < 0.05$ (vs. domperidone), $^{\#}$ $P < 0.05$ (vs. domperidone + quinpirole). Dark columns: adrenal dopamine; hatched columns: plasma adrenaline; stippled columns: heart adrenaline.

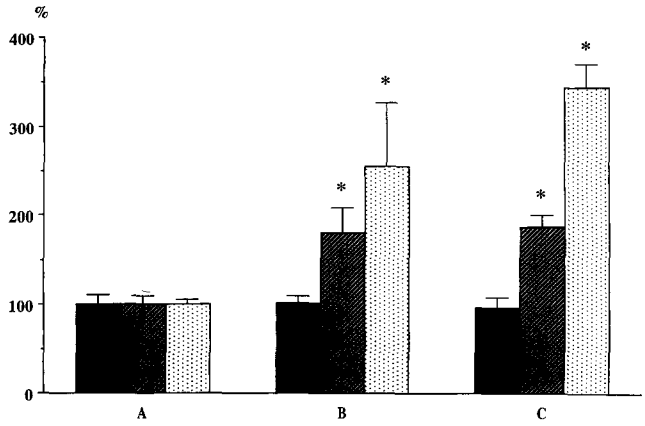

Fig. 3. Effects of the blood sampling procedure, including the preceding operation, on forebrain DOPAC, adrenal dopamine and heart adrenaline in rats. All three groups (A, B and C) were injected twice with saline: 150 and 30 min before decapitation. $n = 4–5$. Group A was not operated (it was under the same experimental conditions as the controls in the experiments without blood sampling); group C was under the same conditions as the control group in the experiments with blood sampling (operation plus blood sampling); group B was operated only, but blood sampling was not performed. The data are shown as the means ± S.E. expressed as percents of the value for the controls, i.e. group A (226 ± 23 ng/g forebrain DOPAC, 112 ± 10 ng/pair adrenal dopamine, 13 ± 1 ng/g heart adrenaline). * $P < 0.05$ (vs. corresponding control). Black columns: forebrain DOPAC; dark columns: adrenal dopamine; stippled columns: heart adrenaline.

dopamine and heart adrenaline following 0.3 mg/kg and higher doses (Fig. 5). These effects of quinpirole (0.3 mg/kg, 30 min) were blocked by domperidone (3 mg/kg, 150 min) ($n = 5$ in each group; values for the saline-treated controls 166 ± 5 ng/pair adrenal dopamine, 20 ± 1 ng/g heart adrenaline).

Also talipexole, a dopamine autoreceptor agonist, which is apparently inactive on postsynaptic dopamine receptors in the absence of a dopamine D_1 receptor agonist (Hjort and Carlsson, 1987), induced a significant increase in heart adrenaline as well as in adrenal dopamine in doses between 0.3 and 3 mg/kg (45 min) (Fig. 6). The adrenal dopamine and heart adrenaline increasing effects of talipexole (0.2 mg/kg, 45 min) were blocked by domperidone (same dose as for quinpirole) ($n = 5$ in each group, values for the saline-treated controls 171 ± 11 ng/pair adrenal dopamine, 22 ± 1 ng/g heart adrenaline).

Apomorphine, a classical non-selective dopamine receptor agonist, was administered in various doses (0.3–10 mg/kg), 30 min (Fig. 7a), or 60 min (Fig. 7b) before decapitation. A statistically significant increase for heart adrenaline was observed only following the highest dose of apomorphine both at 30 and 60 min, although a tendency to an increase was observed even following lower doses especially after 60 min of apomorphine administration. On the other hand, as previously reported, a statistically significant increase in adrenal dopamine was obtained following the lower doses – 0.3 mg/kg (30 min) and 1 mg/kg (60 min) (Kujacic et al., 1990). In the experiments with apomorphine + domperidone reported earlier, however (see Kujacic et al., 1990), a low dose of apomorphine (0.5 mg/kg, 30 min), which had to be used in order to demonstrate blockade of apomorphine effects on adrenal dopamine by domperidone, was not enough to induce a statistically significant increase in heart adrenaline.

The levels of adrenal dopamine and heart adrenaline observed in these experiments with three different dopamine D_2 receptor agonists (quinpirole, talipexole and apomorphine) were plotted against each other, separately for each drug, to investigate if the ratio of adrenal dopamine and heart adrenaline was dependent on the drug used. A lack of dependence, i.e. identical slopes, would indicate some generality of the regression models described above for calculations of the plasma levels adrenaline when different drugs are used, at least under the experimental conditions used here. Thus the slopes for talipexole (0.622; $df = 19$) and apomorphine (0.611; $df = 34$) were within the 95% confidence interval for the slope of quinpirole (0.510–0.898; $df = 52$). To further substantiate this generalisation we also performed a calculation of a PLS regression model without using information about differences in treatments. The outcome of this calculation showed that even with this information omitted, the information about plasma adrenaline is largely retained. The one-component model (based on percent of control data) obtained in this case accounted for 94.6% and 78.2% of the variance in X and Y blocks respectively ($r^2_{cv} = 0.773$, i.e. a significance beyond the 99.9% level) (see Fig. 8).

3.5. Dopamine D_1 receptors are not involved in the changes in adrenal dopamine and heart adrenaline levels

SCH 23390, a dopamine D_1 receptor antagonist, at a dose of 1 mg/kg, 45 min, did not block the apomorphine (5 mg/kg, 30 min)-induced increase in adrenal dopamine and heart adrenaline. SCH 23390 itself did not induce any statistically significant increase in adrenal dopamine and heart adrenaline. The dopamine D_1 receptor agonist, SKF 38393 (20 mg/kg, 30 min), also did not have any significant effect on adrenal dopamine and heart adrenaline (data not shown).

3.6. Dopamine D_2 antagonists do not induce changes in adrenal dopamine and heart adrenaline levels

The dopamine D_2 receptor antagonist, raclopride (5 mg/kg, 120 min), although capable of inducing a statistically significant increase in forebrain DOPAC (almost 3.5-fold compared to control) did not have any signifi-

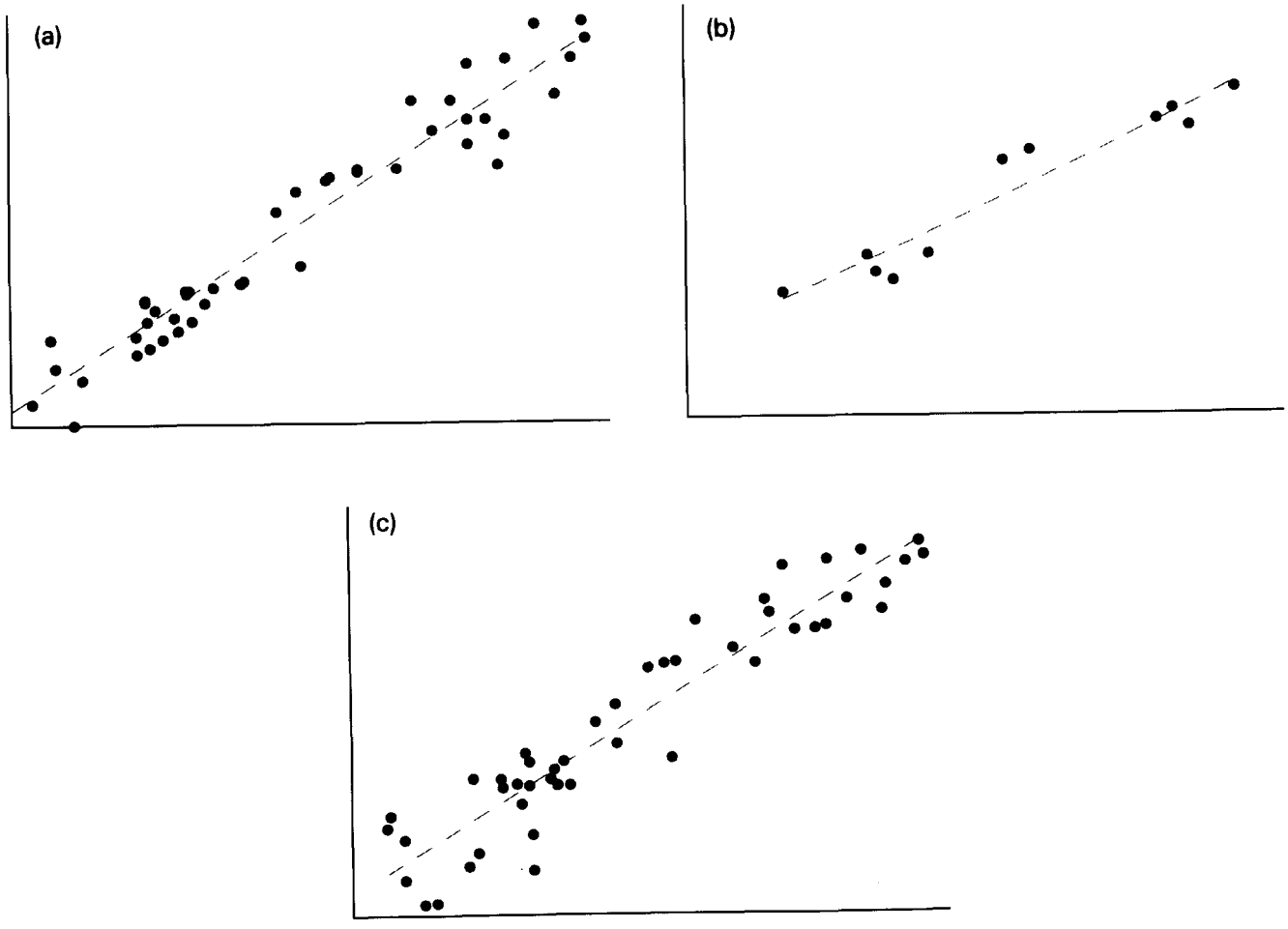

Fig. 4. The relationship between the calculated and the experimentally found arterial plasma levels of adrenaline. Log[plasma adrenaline] was used as the dependent variable. $T_{(1-7)}$ denotes seven different possibilities for treatment used. Abscissa: measured log[plasma adrenaline]; ordinate: calculated (a; c) or predicted (b) log[plasma adrenaline]. (a) The model is based on percent of the values for the saline-treated controls. Regression equation: log[plasma adrenaline] = $1.504 + 1.51 \times 10^{-3} \times$ [adrenal dopamine] + $2.54 \times 10^{-3} \times$ [heart adrenaline] − $0.195 \times T_1 + 1.48 \times 10^{-2} \times T_2 + 7.54 \times 10^{-2} \times T_3 + 0.202 \times T_4 - 0.120 \times T_5 - 2.82 \times 10^{-2} \times T_6 + 0.322 \times T_7$; $r = 0.966$. (b) External predictions of log[plasma adrenaline] for 11 observations not included in the calibration model. The model is based on percent of the values for the saline-treated controls. $r = 0.962$. (c) The model is based on the absolute values. Regression equation: log[plasma adrenaline] = $-2.49 + 0.52 \times$ [adrenal dopamine] + $0.435 \times$ [heart adrenaline] − $0.194 \times T_1 + 0.111 \times T_2 + 0.224 \times T_3 + 0.164 \times T_4 - 0.201 \times T_5 - 0.032 \times T_6 + 0.23 \times T_7$; $r = 0.945$.

cant effect on either adrenal dopamine or heart adrenaline (Fig. 9). Similarly, no significant effects were obtained on adrenal dopamine or heart adrenaline following two doses of raclopride (5 mg/kg each) injected 7 and 3 h before decapitation, but forebrain DOPAC was increased 4.7-fold compared to the control (data not shown).

Domperidone, a dopamine D_2 receptor antagonist which is supposed to have only peripheral effects (in support of this, no changes in forebrain DOPAC were observed, except for a slight, though statistically significant increase following the high dose of 10 mg/kg), administered in doses of 3–10 mg/kg s.c., 150–180 min) did not induce, like raclopride, any statistically significant changes either in adrenal dopamine (with the exception of the 10 mg/kg dose), or in heart adrenaline in seven separate experiments.

4. Discussion

Our previous investigations dealing with the effects of different dopamine D_1- and D_2-like receptor agonists and antagonists on the dopamine levels in rat adrenal glands suggested that stimulation of dopamine D_2-like receptors with dopamine receptor agonists yields an increase in adrenal dopamine levels. The blockade by domperidone (Kujacic et al., 1990,1991) and failure of i.c.v. administered dopamine to induce any adrenomedullary changes (Kujacic and Carlsson,

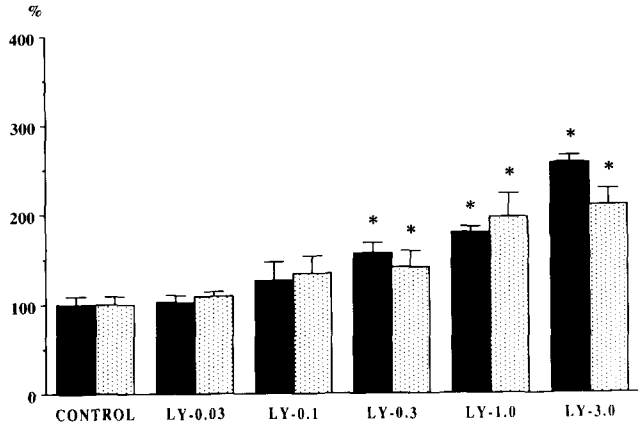

Fig. 5. Dose-dependent changes in adrenal dopamine and heart adrenaline induced by quinpirole in rats without blood sampling. $n = 4$ in each group. Quinpirole (LY) in doses of 0.03–3 mg/kg was administered s.c., 30 min before decapitation. The data are shown as the means ± S.E. expressed as percent of the value for the saline-treated controls (187 ± 18 ng/pair adrenal dopamine, 26 ± 2 ng/g heart adrenaline). Data for the adrenal dopamine were reported previously (Kujacic et al., 1990). * $P < 0.05$ (vs. corresponding control). Dark columns: adrenal dopamine; stippled columns: heart adrenaline.

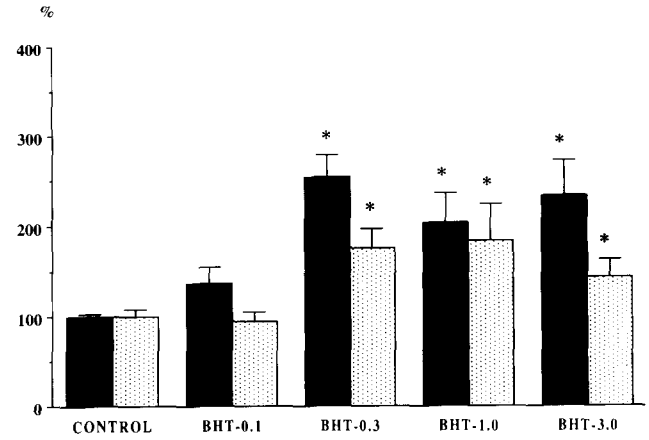

Fig. 6. Dose-dependent changes in adrenal dopamine and heart adrenaline induced by talipexole in rats without blood sampling. $n = 4$ in each group. Talipexole (BHT) in doses of 0.1–3 mg/kg was administered s.c., 45 min before decapitation. The data are shown as the means ± S.E. and expressed as percent of the value for the saline-treated controls (201 ± 6 ng/pair adrenal dopamine; 26 ± 2 ng/g heart adrenaline). Data for adrenal dopamine were previously reported (Kujacic et al., 1991). * $P < 0.05$ (vs. corresponding control). Dark columns: adrenal dopamine; stippled columns: heart adrenaline.

1994) would indicate peripheral localisation of these receptors. The variations in adrenal dopamine levels were shown to reflect changes in catecholamine synthesis, as mentioned in the Introduction section. The role of these peripheral dopamine D_2-like receptors thus seems to differ from that of the forebrain dopamine autoreceptors, at least in the rat: the latter exert an inhibitory function, including inhibition of dopamine synthesis in forebrain dopamine neurones.

The present data suggest that dopamine D_2-like receptor agonists are also capable of stimulating adrenaline release from the adrenal medulla in vivo.

As generally accepted, plasma levels of adrenaline accurately reflect the release of adrenaline from the adrenal medulla. However, as mentioned earlier, in vivo manipulations such as implantation of venous and arterial catheters and blood sampling itself, in spite of all precautionary measures, induce a certain amount of stress, which is reflected in a distinct and statistically significant increase both in adrenal dopamine and heart adrenaline (see Fig. 3), and consequently probably in

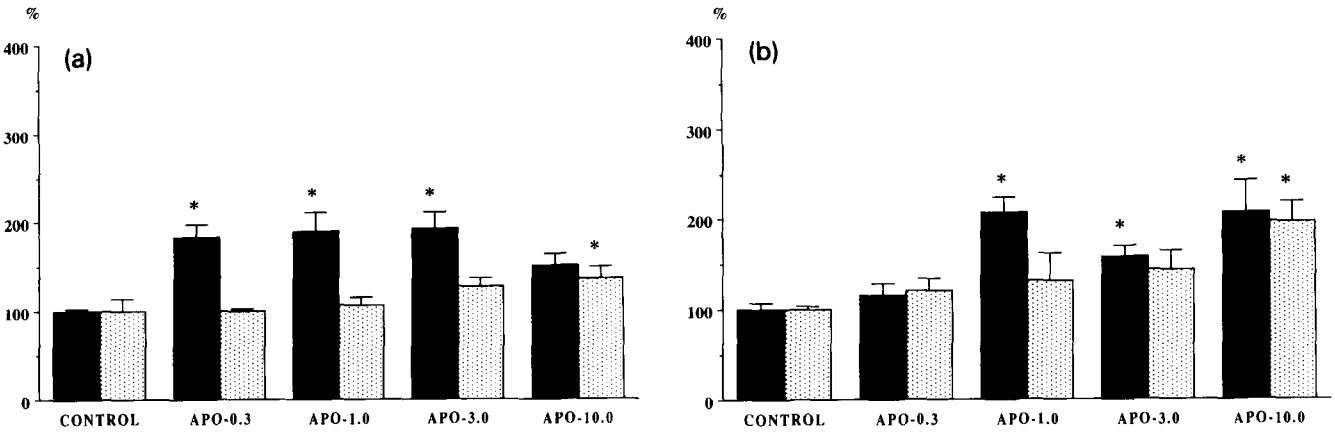

Fig. 7. Dose-dependent changes in adrenal dopamine and heart adrenaline induced by apomorphine in rats, without blood sampling. Data were obtained in two separate experiments (a, b). $n = 4$–5. Apomorphine (APO) in doses of 0.3–10 mg/kg s.c. was administered 30 min (a), or 60 min (b) before decapitation. Data are shown as the means ± S.E. expressed as percent of the value for the saline-treated controls (a: 179 ± 5 ng/pair adrenal dopamine, 39 ± 5 ng/g heart adrenaline; b: 170 ± 15 ng/pair adrenal dopamine, 31 ± 1 ng/g heart adrenaline). Data for the adrenal dopamine were previously reported (Kujacic et al., 1990). * $P < 0.05$ (vs. corresponding control). Dark columns: adrenal dopamine; stippled columns: heart adrenaline.

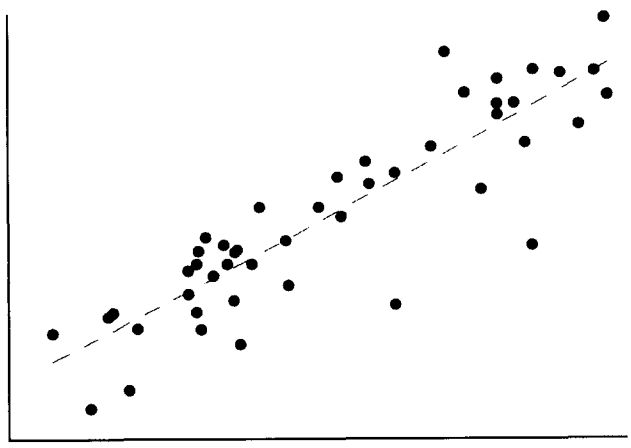

Fig. 8. Calibration model where information about differences in treatment is omitted. The model is based on percent of control data. Regression equation: log[plasma adrenaline] = $1.48 + 2.43 \times 10^{-3} \times$ [adrenal dopamine] + $3.71 \times 10^{-3} \times$ [heart adrenaline]; $r = 0.885$.

an increase in plasma adrenaline levels. On the other hand, as suggested by Carlsson and Carlsson (1989), the heart adrenaline level, although providing only indirect evidence that adrenaline is released from the adrenal medulla and subsequently taken up by the catecholamine transporter of the sympathetic nerve terminals in the heart, may be a useful, at least qualitative, indicator. Furthermore, by using PLS regression, we developed a model (as explained in detail in the Results section), which made it possible to use an indirect approach to estimate the plasma levels of adrenaline. This was accomplished by taking adrenal dopamine, heart adrenaline and information about treatment as predictors of plasma adrenaline levels. It could be argued that similar results could be obtained by means of multiple regression. However, the serious shortcomings of multiple regression, with respect to reliability, compared to PLS (Topliss and Edwards, 1979; Clark and Cramer, 1993) made us choose the latter method. Thus, the calibration model developed for quinpirole (see Fig. 4c) could be more generally used for approximate prediction of plasma adrenaline levels following different treatments (in rats and under experimental conditions similar to ours) if the slopes of the regression lines fit into the confidence interval of 95% of the slope of the regression line for quinpirole. Should this not be the case, it is necessary to develop a separate calibration model, using the same technique as described in the Results section. Any shortcoming of this indirect method probably appears only if the drug in question interferes with adrenaline transport.

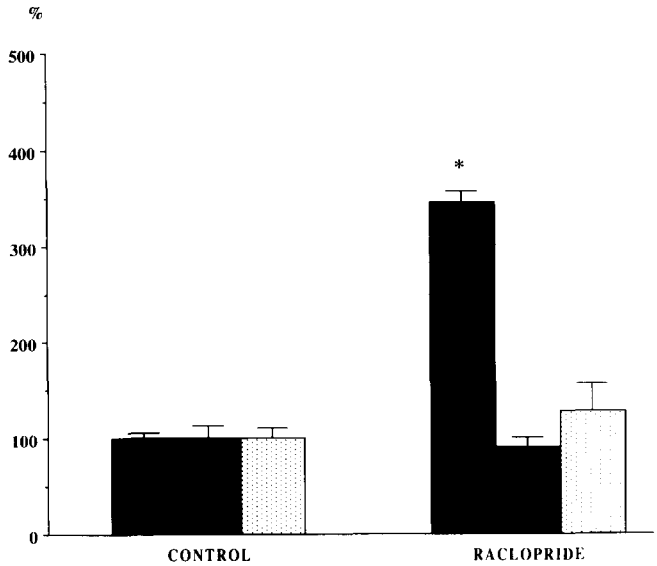

Fig. 9. Changes in forebrain DOPAC, adrenal dopamine and heart adrenaline induced by raclopride. $n = 5$ in each group. Raclopride, 5 mg/kg was administered 2 h before decapitation. The data are shown as the means ± S.E. expressed as percent of the value for the saline-treated controls (306 ± 15 ng/g forebrain DOPAC, 161 ± 20 ng/pair adrenal dopamine, 18 ± 2 ng/g heart adrenaline). * $P < 0.05$ (vs. corresponding control). Black columns: forebrain DOPAC; dark columns: adrenal dopamine; stippled columns: heart adrenaline.

As already mentioned, adrenaline release was also increased in control groups as a consequence of the blood sampling procedure and the 'normal' values reported in the literature are consequently higher than they are in reality. Accordingly, the calculated values shown in Table 1 for arterial plasma adrenaline in the saline-treated groups probably represent real basal levels of plasma adrenaline in rats. This is the first time such estimates have been published.

Our results with domperidone suggest that the dopamine receptors involved in the stimulation of adrenaline release are at least partly located peripherally. According to Regunathan et al. (1989), however, the stimulating effects of piribedil and talipexole on adrenaline release in the rat were blocked by splanchnicectomy suggesting a central mechanism of action. Different possibilities may be considered to reconcile these apparently discrepant observations. Tonic stimulation of the secretory nerves to the adrenal medulla may play a permissive role for a peripherally mediated dopaminergic effect on adrenomedullary secretion. Alternatively, the dopamine receptors involved in the control of adrenomedullary secretion may be located centrally, but outside the blood-brain barrier, or peripherally but acting via a reflex on the adrenomedullary secretion.

The absence of an effect, or the existence of an at most marginal effect of dopamine receptor antagonists on adrenal dopamine levels (with exception of a statistically significant effect of 10 mg/kg of domperidone) and heart adrenaline levels suggests that the dopamine receptors involved are not, or at most slightly, tonically stimulated. This seems to be the case even under conditions of stress-induced elevation of adrenomedul-

lary activity, as suggested by the results of our present experiments with catheters implanted into the carotid artery and jugular vein.

Furthermore, the possibility of some kind of reflex-mediated stimulation of adrenal medulla cannot be excluded, although a reflex reaction to hypotension is unlikely, as under experimental conditions similar to ours acute administration of quinpirole to conscious normotensive unrestrained rats induced an acute pressor effect and at the same time increased plasma noradrenaline and adrenaline levels (Nagahama et al., 1986).

It is unlikely that the receptors studied by us are identical to the dopamine receptors occurring in the adrenomedullary cells. These receptors appear to have an inhibitory action on the secretion of adrenomedullary hormones (see Introduction), whereas the receptors discovered by us are stimulatory. Possibly, the former receptors are not important in the intact system, at least not in the rat, and the location of the latter remains an open question.

In conclusion, we have found an acute stimulating effect of dopamine D_2-like receptor agonists on adrenaline release from the adrenal medulla in the rat, mediated via most likely peripherally located dopamine receptors which, however, seem to be different from the dopamine receptors identified on adrenal chromaffin cells in vitro.

Acknowledgements

The authors are grateful to the late Professor Jan Häggendal for useful discussions and suggestions regarding the collection of blood samples and the determination of catecholamines in plasma and for generously placing his resources at our disposal. The skilful technical assistance of Mrs. Lena Löfberg, Mrs. Arja Schedwin and Mrs. Monika Lindhé-Fredriksen is also gratefully acknowledged. This investigation was financially supported by The Upjohn Company, Michigan, USA, the Wilhelm and Martina Lundgrens Foundation, the Medical Faculty University of Göteborg and the Anna Ahrenbergs Foundation.

References

Artalejo, A.R., A.G. Garcia, C. Montiel and P. Sanchez-Garcia, 1985, A dopaminergic receptor modulates catecholamine release from the cat adrenal gland, J. Physiol. 362, 359.

Artalejo, C.R., M.A. Ariano, R.L. Perlman and A.P. Fox, 1990, Activation of facilitation calcium channels in chromaffin cells by D_1 dopamine receptors through a cAMP/protein kinase A-dependent mechanism, Nature 348, 239.

Bigornia, L., M. Suozzo, K.A. Ryan, D. Napp and A.S. Schneider, 1988, Dopamine receptors on adrenal chromaffin cells modulate calcium uptake and catecholamine release, J. Neurochem. 51, 999.

Bigornia, L., C.N. Allen, C.-R. Jan, R.A. Lyon, M. Titeler and A.S. Schneider, 1990, D_2 dopamine receptors modulate calcium channel currents and catecholamine secretion in bovine adrenal chromaffin cells, J. Pharmacol. Exp. Ther. 252, 586.

Carlsson, M. and A. Carlsson, 1989, Effects of mild stress on adrenal and heart catecholamines in male and female rats, J. Neural Transm. 77, 217.

Clark, M. and R.D. Cramer, III, 1993, The probability of chance correlations using partial least squares (PLS), Quant. Struct.-Act. Relat. 12, 137.

Collett, A.R. and D.F. Story, 1982, Is catecholamine release from the rabbit adrenal gland subject to regulation through dopamine receptors or β-adrenoceptors?, Clin. Exp. Pharmacol. Physiol. 9, 436.

Dunn, III, W.J. and S. Wold, 1990, Pattern recognition techniques in drug design, in: Comprehensive Medicinal Chemistry, eds. C. Hansch, P.G. Sammes and J.B. Taylor (Pergamon Press, New York) p. 691.

Foucart, S., P. Lacaille-Belanger, T. Kimura, R. Nadeau and J. De Champlain, 1988, Modulation of adrenal catecholamine release by D_2 dopamine receptors in the anaesthetized dog, Clin. Exp. Pharmacol. Physiol. 15, 601.

Gonzalez, M.C., A.R. Artalejo, C. Montiel, P.P. Hervas and A.G. Garcia, 1986, Characterization of a dopaminergic receptor that modulates adrenomedullary catecholamine release, J. Neurochem. 47, 382.

Hjorth, S. and A. Carlsson, 1987, Postsynaptic dopamine (DA) receptor stimulator properties of the putative DA autoreceptor-selective agonist B-HT 920 uncovered by co-treatment with the D-1 agonist SK&F 38393, Psychopharmacology 93, 534.

Huettl, P., G.A. Gerhardt, M.D. Browning and J.M. Masserano, 1991, Effects of dopamine receptor agonists and antagonists on catecholamine release in bovine chromaffin cells, J. Pharmacol. Exp. Ther. 257, 567.

Kujacic, M. and A. Carlsson, 1993, Evidence for an increased catecholamine synthesis in rat adrenal glands following stimulation of peripheral dopamine receptors, J. Neural Transm. [GenSect] 92, 73.

Kujacic, M. and A. Carlsson, 1994, I.c.v. dopamine fails to alter adrenomedullary function in rats, J. Neural Transm. [GenSect] 95, 235.

Kujacic, M., K. Svensson, L. Löfberg and A. Carlsson, 1990, Acute changes in dopamine levels in rat adrenal gland after administration of dopamine receptor agonists and antagonists, Eur. J. Pharmacol. 177, 163.

Kujacic, M., K. Svensson, L. Löfberg and A. Carlsson, 1991, Dopamine receptors, controlling dopamine levels in rat adrenal glands – comparison with central dopaminergic autoreceptors, J. Neural Transm. [GenSect] 84, 195.

Lyon, R.A., M. Titeler, L. Bigornia and A.S. Schneider, 1987, D2 dopamine receptors on bovine chromaffin cell membranes: identification and characterization by (^{3}H)*N*-methylspiperone binding, J. Neurochem. 48, 631.

Mannelli, M., C. Pupilli, G. Fabbri, R. Musante, M.L. De Feo, F. Franchi and G. Guisti, 1988, Endogenous dopamine (DA) and DA2 receptors: a mechanism limiting excessive sympathetic-adrenal discharge in humans, J. Clin. Endocrinol. Metab. 66, 626.

Mannelli, M., R. Lanzillotti, L. Ianni, C. Pupilli and M. Serio, 1990, Dopaminergic modulation of human adrenal medulla: indirect evidence for the involvement of DA-2 receptors located on chromaffin cells, J. Auton. Pharmacol. 10 (Suppl. 1), s79.

Mercuro, G., G. Gessa, C.A. Rivano, L. Lai and A. Cherchi, 1988, Evidence for a dopaminergic control of sympathoadrenal catecholamine release, Am. J. Cardiol. 62, 827.

Milliken, G.A. and D.E. Johnson, 1984, Analysis of Messy Data. Vol. I: Designed Experiments (Lifetime Learning Publications, Belmont, CA) p. 33.

Montiel, C., A.R. Artalejo, P.M. Bermejo and P. Sanchez-Garcia, 1986, A dopaminergic receptor in adrenal medulla as a possible site of action for the droperidol-evoked hypertensive response, Anesthesiology 65, 474.

Nagahama, S., Y.-F. Chen, M.D. Lindheimer and S. Oparil, 1986, Mechanism of the pressor action of LY 171555, a specific dopamine D2 receptor agonist, in the conscious rat, J. Pharmacol. Exp. Ther. 236, 735.

Quik, M., L. Bergeron, H. Mount and J. Philie, 1987, Dopamine D2 receptor binding in adrenal medulla: characterization using (^{3}H)spiperone, Biochem. Pharmacol. 36, 3707.

Regunathan, S., K. Missala and T.L. Sourkes, 1989, Central regulation of adrenal tyrosine hydroxylase: effect of induction on catecholamine levels in the adrenal medulla and plasma, J. Neurochem. 53, 1706.

Schalling, M., Å. Dagerlind, M. Goldstein, M. Ehrlich, P. Greengard and T. Hökfelt, 1990, Comparison of gene expression of the dopamine D2 receptor and DARPP-32 in rat brain, pituitary and adrenal gland, Eur. J. Pharmacol. Mol. Pharmacol. 188, 277.

Sontag, J.-M., P. Sanderson, M. Klepper, D. Aunis, K. Takeda and M.-F. Bader, 1990, Modulation of secretion by dopamine involves decreases in calcium and nicotinic currents in bovine chromaffin cells, J. Physiol. 427, 495.

Stone M., 1974, Cross-validatory choice and assessment of statistical predictions, J. R. Stat. Soc. B36, 111.

Topliss, J.G. and R.P. Edwards, 1979, Chance factors in studies of quantitative structure-activity relationships, J. Med. Chem. 22, 1238.

Wold, H., 1975, Soft modelling by latent variables; the non-linear iterative partial least squares (NIPALS) approach, in: Perspectives in Probability and Statistics, Papers in Honour of M.S. Bartlett, ed. J. Gani (Academic Press, New York) p. 117.

Wold S., 1978, Cross-validatory estimation of the number of components in factor and principal component models, Technometrics 20, 397.

Wold, S., C. Albano, W.J. Dunn, III, U. Edlund, K. Esbensen, P. Geladi, S. Hellberg, E. Johansson, W. Lindberg and M. Sjöström, 1984, Multivariate data analysis in chemistry, in: NATO Adv. Study in Chemometrics, ed. B.R. Kowalski (Reidel Publ. Co., Dordrecht) p. 17.