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Extracellular Catecholamine Levels in Rat Hippocampus after a Selective *Alpha*-2 Adrenoceptor Antagonist or a Selective Dopamine Uptake Inhibitor: Evidence for Dopamine Release from Local Dopaminergic Nerve Terminals[1]

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ABSTRACT

The effect of 6-chloro-2,3,4,5-tetrahydro-3-methyl-1-H-3-benzazepine (SKF 86466), a selective nonimidazoline *alpha*-2 adrenoceptor antagonist, on hippocampal release of norepinephrine and dopamine in conscious rats was investigated by *in vivo* microdialysis and high-pressure liquid chromatography. Additionally, extracellular concentrations of hippocampal dopamine (DA) and norepinephrine (NE), during infusion of selective monoamine uptake inhibitors, were determined in freely moving rats. The basal concentration of NE in the dialysate was 4.9 ± 0.3 pg/20 μl. Intravenous administration of 5 or 10 mg/kg of SKF 86466 was associated with a transient increase (30 min) of 2-fold (12 ± 1 pg/20 μl; $P < .05$) and 8-fold (39 ± 3 pg/20 μl; $P < .05$), respectively, in dialysate NE, whereas a 1-mg/kg dose had no effect. DA was not detected in basal dialysates, but after the administration of 5 or 10 mg/kg of SKF 86466, 3.9 ± 0.4 and 6.4 ± 0.6 pg/20 μl, respectively, was present in the dialysates. The maximum increase in dialysate DA was reached 60 to 90 min after SKF 86466. The DA was not derived from plasma because plasma NE was elevated after the 5 mg/kg dose of SKF 86466 whereas no plasma DA was detected. In order to determine whether DA was present in noradrenergic nerve terminals, the dopamine β-hydroxylase inhibitor SKF 102698 was administered (50 mg/kg i.p.). The inhibitor decreased dialysate NE but DA was still not detected in the dialysate. When SKF 86466 (5 mg/kg i.v.) was administered 4 hr after SKF 102698, DA appeared in the dialysate but there was no increase in dialysate NE. Administration through the dialysis probe of the DA uptake inhibitor, GBR-12909 (0.1 and 1 μM), dose-dependently increased DA levels to 5.7 ± 1.2 and 9.6 ± 2.8 pg/20 μl, respectively. GBR-12909 had no effect on hippocampal NE. Desipramine (5 and 10 μM) increased dose-dependently dialysate NE and increased DA concentrations to detectable levels (2.7 ± 0.5 and 3.5 ± 0.7 pg/20 μl, respectively). These results suggest that the *alpha*-2 adrenoceptors modulate both NE and DA release in the rat hippocampus and that DA detected in the hippocampal dialysate might be released from dopaminergic neurons.

The function of the presynaptic *alpha*-2 adrenoceptors at central noradrenergic junctions has been demonstrated largely by *in vitro* studies. Thus, it is well established that *alpha*-2 adrenergic agonists inhibit the release of NE from nerve endings (Langer, 1981; Schoffelmeer and Mulder, 1983; Dubocovich, 1984; Jackisch *et al.*, 1984), whereas *alpha*-2 adrenergic antagonists enhance the release of NE (Dubocovich, 1984; Jackisch *et al.*, 1984). These observations support a role for presynaptic *alpha*-2 autoreceptor-mediated inhibition in the physiological regulation of NE release. The hippocampus receives a moderate NE innervation from the nucleus locus coeruleus *via* the fimbria-fornix, cingulus and the ventral amygdaloid bundles (Foote *et al.*, 1983; Swanson *et al.*, 1987; Oleskevish *et al.*, 1989), and appears to have at least four subtypes

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ABBREVIATIONS: NE, norepinephrine; DA, dopamine; SKF 86466, 6-chloro-2,3,4,5-tetrahydro-3-methyl-1-H-3-benzazepine; GBR-12909, 1-[2-[bis(4-fluorophenyl)methoxy]-ethyl]-4-(3-phenylpropyl)piperazine dihydrochloride; DHBA, 3,4-dihydroxybenzylamine; HPLC, high-pressure liquid chromatography; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; SKF 102698, 1-(3,5-difluorobenzyl)imidazole-2-thione; ANOVA, analysis of variance.

of adrenoceptors (*alpha*-1, *alpha*-2, *beta*-1 and *beta*-2) (Swanson *et al.*, 1987). Furthermore, the hippocampus receives dopaminergic afferents from both A9 and A10 cell groups in the ventral tegmental area (Scatton *et al.*, 1980), but the DA concentration in hippocampus is low (Bischoff *et al.*, 1979; Verney *et al.*, 1985), and it has been considered only to represent a NE precursor pool. However, there is evidence that DA acts as a neurotransmitter in hippocampus, independently of noradrenergic neurons (Bischoff *et al.*, 1979; Ishikawa *et al.*, 1982; Verney *et al.*, 1985).

Extracellular levels of monoamines in the hippocampus have been studied by *in vivo* microdialysis techniques (Abercrombie *et al.*, 1988; Kalén *et al.*, 1988; Abercrombie and Zigmond, 1989; Globus *et al.*, 1989). Stimulation of the presynaptic *alpha*-2 adrenoceptors inhibited the hippocampal release of NE whereas *alpha*-2 adrenoceptor antagonists increased release (Abercrombie *et al.*, 1988). We monitored catecholamine release in hippocampal dialysates of the conscious rat after systemic administration of the selective *alpha*-2 adrenoceptor blocker SKF 86466. SKF 86466 is a cyclic derivative of phenylethylamine and a selective *alpha*-2 adrenoceptor antagonist (Hieble *et al.*, 1986; Daly *et al.*, 1988). Unlike idazoxan, SKF 86466 does not bind to imidazole sites in the rat brain (Ernsberger *et al.*, 1990). Furthermore, SKF 86466 does not significantly interact with dopaminergic, *beta* adrenergic, serotonin or muscarinic receptors (Hieble *et al.*, 1986). This drug has no known effect on dopamine metabolism and a very weak affinity for DA receptors (the IC_{50} of SKF 86466 for [^{3}H]spiroperidol binding in rat brain homogenates and for DA-sensitive adenylate cyclase in rat caudate is 10 μM (Dr. P. Hieble, personal communication). In our preliminary studies, SKF 86466 increased hippocampal NE release in a dose-dependent manner. We also detected DA in the hippocampal dialysate of SKF 86466-treated rats. To elucidate the functional significance of *alpha*-2 adrenoceptors in hippocampal adrenergic tone, we studied DA and NE release into hippocampal dialysates obtained under basal conditions and after *alpha*-2 adrenergic blockade by intravenous administration of SKF 86466 in conscious rats. To elucidate further the source of DA in the hippocampal dialysate, the animals were treated with a DA β-hydroxylase inhibitor before SKF 86466 administration. In addition, we determined whether selective local inhibition of the DA transporter would result to DA release from dopaminergic nerves projecting to the hippocampus. For this purpose, we monitored hippocampal extracellular catecholamine levels during local intrahippocampal infusion through the dialysis tubing of GBR-12909, a selective DA uptake inhibitor (Heikkila and Manzino, 1984; Andersen, 1989). For comparison, the catecholamine release was also monitored during infusion of the NE uptake inhibitor, desipramine (Heikkila and Manzino, 1984).

Material and Methods

Male Sprague-Dawley rats (260–340 g) were used in all experiments. After the surgical operations, the rats were housed individually in plastic cages (21 × 27 × 16 cm, W × L × H) with food and water *ad libitum*. In the end of the experiment they were sacrificed with an overdose of pentobarbital.

Dialysis Loop Preparation and Calibration

A dialysis loop was constructed from a 1.0-cm piece of hollow dialysis fiber with a molecular weight cutoff of 5,000 (Nephrosis Allegro H.F., Organon Teknika Corp, Durham, NC). A 1.5-cm length of nichrome wire (A-M System) was inserted into the fiber. Using the wire as a guide, each end of the dialysis tubing was inserted into a 2.5-cm length of capillary tubing (All Tech Associates Inc., Deerfield, IL) to the depth of 2.0 mm and glued into place with cyanoacrylate adhesive (Elmer's Wonderbond Plus). After the adhesive had dried, the dialysis fiber was bent into a loop. A 16-gauge stainless steel tubing was placed over the free ends of the capillary tubing and positioned just 2 mm above the dialysis loop, where it was glued with adhesive cyanoacrylate allowing 4 mm exchangeable surface for dialysis.

The *in vitro* recovery of DA and NE were determined for each probe as follows. Filtered artificial cerebrospinal fluid (147 mM NaCl, 4.0 mM KCl and 2.30 mM $CaCl_2$; pH adjusted to 7.0 with $NaHCO_3$) was perfused through the probe at 1 μl/min with a CMA/100 microinjection pump. The recovery was assessed in a beaker of the artificial cerebrospinal fluid solution to which 10 ng/ml standards of DA and NE had been added. The amount of DA and NE in the perfusates were directly compared to that in the buffer. The *in vitro* recoveries were 24.6 ± 1% for DA and 25.0 ± 3% for NE. The values reported here are not corrected for these recoveries.

Studies with the *Alpha*-2 Adrenoceptor Antagonist, SKF 86466

Animal procedures. The rats were anesthetized with an intramuscular injection of ketamine (130 mg/kg) and acepromazine (1.3 mg/kg). In experiments in which plasma was sampled, PE-50 catheters were inserted into the left femoral vein and artery (plasma sampling experiment only). The catheters were tunneled under the back skin and exteriorized at the nape of the neck. The rat was then mounted on a stereotaxic apparatus (David Kopf Inc., Tujunga, CA). The skull was exposed and a small hole was drilled to allow implantation of the dialysis probe into the dorsal hippocampus using the coordinates: anteroposterior, −4.2 mm; mediolateral, −2.0 mm relative to bregma and vertical, −4.0 mm relative to dura with incisor bar set at −3 mm from horizontal zero. The dialysis probe was fixed on the skull with two screws and dental cement and artificial cerebrospinal fluid was continuously perfused through the probe at a rate of 1 μl/min. The animal was allowed to recover for 4 hr after the implantation and perfusate was collected in conscious freely moving rats in 30-min fractions into a mini-vial containing 30 pg of DHBA in 6 μl of 0.1 N perchloric acid. The fractions were collected before and after femoral vein injection of either saline or SKF 86466.

Experimental protocols. All treatment groups were independent and each rat received only one injection of saline, artificial cerebrospinal fluid or one dose level of drugs.

Dose-response studies. After collection of two 30-min base-line samples, saline or 1, 5 or 10 mg/kg of SKF 86466 was injected intravenously at 120-min intervals. The perfusate was collected at 30-min intervals after saline or drug administration.

Time-response studies. After collection of the two base-line samples, saline, SKF 86466 at doses of 5 or 10 mg/kg or idazoxan at doses 1 or 5 mg/kg was injected intravenously and 30-min portions of the perfusate were collected for 240 min.

Plasma sampling. In a separate group of rats, blood specimens were collected simultaneously with the perfusate samples. The blood samples (0.8 ml) were withdrawn from the arterial catheter at 30-min intervals after the administration of SKF 86466 (5 mg/kg i.v.). The blood withdrawn was replaced with an equal volume of fresh rat blood. The blood specimens were collected in chilled test tubes, centrifuged (Beckman microfuge B) immediately for 1 min and the plasma removed and rapidly frozen on dry ice. NE and DA were separated by alumina extraction and assayed by HPLC as described below.

Treatment with the dopamine β-hydroxylase inhibitor. The dopamine β-hydroxylase inhibitor SKF 102698 (50 mg/kg) or vehicle (10% dimethyl sulfoxide in distilled water) was injected i.p. 4 hr before the i.v. injection of SKF 86466 (5 mg/kg). The hippocampal perfusate and plasma samples were collected at 30-min intervals during the experiment and processed as described above.

Studies with Hippocampal Infusion of DA or NE Uptake Inhibitors

The rats were anesthetized with an intramuscular injection of ketamine (130 mg/kg) and acepromazine (1.3 mg/kg) and the microdialysis probe guide cannulas were implanted 20 to 24 hr before the experiments using stereotaxic coordinates anteroposterior, −4.2 mm and mediolateral, −2.0 mm relative to bregma. Vertical depth was 4.0 mm relative to the dural surface.

In the beginning of the experiment a microdialysis probe (CMA/12, 2 mm membrane, Bioanalytical Systems, Inc., West Lafayette, IN) was inserted in the hippocampus through the guide cannula and perfused with artificial cerebrospinal fluid at a rate of 1 μl/min for 2 hr before starting the collection of perfusate in 30-min fractions. One base-line sample was collected before drug administration. GBR-12909 (0.1 or 1.0 μM) and desipramine hydrochloride (5 or 10 μM) were perfused through the microdialysis probe for 4 hr. Three samples were collected after cessation of drug infusion. The control group was perfused with artificial cerebrospinal fluid.

The rats were allowed to move freely during experiments. In the end of the experiment they were sacrificed with an overdose of pentobarbital.

Analysis of dialysis samples. The dialysate (20 μl) was injected into an HPLC system equipped with a 7 cm × 4.6 mm 3 μM reverse phase ODS column, an amperometric detector and a glassy carbon electrode (Water's 460 Electrochemical detector, Milford, MA) set at 0.60 V relative to a Ag-AgCl reference electrode. The mobile phase contained 50 mM sodium acetate, 20 mM citric acid, 2 mM octane sulphonate, 0.1 mM ethylenediaminetetraacetic acid, 0.1 mM butylamine and 10% (v/v) methanol, at pH 4.7. The mobile phase was filtered through a 0.22 μM filter and degassed under vacuum by means of ultrasonic agitation. All separations were performed isocratically at a flow rate of 1 ml/min. The temperature of the column was kept at 38°C by means of a column heater. DA and NE concentrations were corrected for the recovery of DHBA and were expressed as pg/20 μl of perfusate.

The sensitivity of the method was estimated from the amount of the compound which gives a peak height 2 times that of base line and was 2 pg for DA and 1.5 pg for NE. The authenticity of DA and NE in the samples was verified by parallel chromatographic and radioenzymatic assays of selected dialysate specimens (table 1). Catecholamine concentrations were measured by a catechol-O-methyltransferase-based radioenzymatic technique (Da Prada and Zürcher, 1976).

In the intrahippocampal infision experiments, DA, NE, DOPAC and HVA concentrations in the dialysate were analyzed using HPLC with electrochemical detection (Waters 460) as described above. In these studies, the mobile phase was slightly modified to improve separation and increase sensitivity, and contained sodium acetate 80 mM, octyl sodium sulphate 0.90 mM, EDTA 0.1 mM, citric acid 70 mM and acetonitrile 4.9% (v/v), pH 2.9 to 3.0. All separations were made isocratically at a flow rate of 1 ml/min at 38°C. The sensitivity of the system was estimated as the amount of the compound giving a peak height 2 times that of base-line noise, and was 2.0 pg for DA, 1.0 pg for NE and DOPAC and 4.0 pg for HVA. *In vitro* recoveries (%) were 25 ± 1 for DA, 25 ± 2 for NE, 28 ± 2 for DOPAC and 33 ± 2 for HVA. The results are not corrected for these recoveries.

TABLE 1

Comparison of dopamine and norepinephrine concentrations in the hippocampal dialysates measured in parallel by either high-pressure liquid chromatography (HPLC) or radioenzymatic assay (REA)

A 90-μl volume of perfusate was collected after intravenous injection of SKF 86466 (5 mg/kg). Thirty microliters was injected into the HPLC system, and the remaining 60 μl was assayed with radioenzymatic method. Abbreviations: BLD, below the limit of detection (1.5 pg/20 μl).

Sample	Dopamine		Norepinephrine	
	HPLC	REA	HPLC	REA
1	4.0	5.2	2.3	1.1
2	2.4	2.8	2.6	2.8
3	2.1	2.3	BLD	0.3
4	3.1	3.1	4.5	3.0

Histology

The brains were quickly removed and frozen on dry ice. Coronal sections (50 μM) of forebrain were cut on a cryostat and stained with 0.1% thionin. Histological localization of the microdialysis probe was verified according to a stereotaxic atlas (Paxinos and Watson, 1986). Our previous studies demonstrated only minimal damage of hippocampal neuronal cell bodies 24 hr after implantation of the loop probe (Shuaib *et al.*, 1990).

Drugs Used

The selective *alpha*-2 adrenoceptor antagonists, SKF 86466 (Hieble *et al.*, 1986; Daly *et al.*, 1988) and idazoxan [2-(2-(1,4 benzodioxanyl))2-imidazoline hydrochloride; Doxey *et al.*, 1983] were dissolved in saline. SKF 102698, a competitive and selective dopamine β-hydroxylase inhibitor (Berkowitz *et al.*, 1988), was dissolved in 10% dimethyl sulfoxide in distilled water. GBR-12909, a selective blocker of DA uptake (Heikkila and Manzino, 1984; Andersen, 1989), and the NE uptake inhibitor, desipramine hydrochloride (Heikkila and Manzino, 1984) were dissolved in artificial cerebrospinal fluid. The pH of the drug solutions was 6 to 7.

Statistical Analysis of the Data

Data in text and figures are represented as mean ± S.E.M. for the indicated number of rats. For statistical analysis the CSS:statistica software package (StatSoft, Inc., Tulsa, OK) was used. Statistics were calculated using two-way ANOVA with repeated measures, one-way ANOVA with a post-hoc Student-Newman-Keul's test or for data with unequal variances the nonparametric Kruskal-Wallis ANOVA with Mann-Whitney U-test or the Wilcoxon matched pairs test. For statistical purposes, when DA and NE concentrations were below detection limit, values of 1.99 and 1.49 pg were used, respectively.

Results

The basal amount of NE averaged 4.4 ± 0.5 pg/20 μl of perfusion. This basal level was not changed by the lower dose of 1 mg/kg dose of SKF 86466. SKF 86466 at doses of 5 and 10 mg/kg increased extracellular levels of DA and NE in the rat hippocampus dose-dependently (fig. 1). Administration of 5 and 10 mg/kg of SKF 86466, was associated with an increase in dialysate NE of about 2-fold (11 ± 1 pg; $P < .05$) and 9-fold (39 ± 3 pg; $P < .05$), respectively. DA was not detected in base-line samples but after the administration of 5 or 10 mg/kg of SKF 86466, dialysate DA concentrations were 3.9 ± 0.4 and 6.4 ± 0.6 pg, respectively (fig. 1). An increase in DA level (6.4 ± 3.5 pg; $P < .05$) was also seen after intravenous administration of idazoxan (5 mg/kg; $n = 3$).

The time course of the increase in dialysate DA and NE after the administration of SKF 86466 is shown in figure 2. The increase in NE reached a maximum in the first 30-min fraction after drug administration and then returned to base line within 90 to 120 min. By contrast, the maximum increase in dialysate DA was reached 90 min after administration of the 5 mg/kg dose of SKF 86466 and subsided in 150 min. After the 10 mg/kg dose, the peak increase was achieved 60 min after drug administration and DA was detectable at least 300 min.

Effect of SKF 86466 on plasma catecholamines. Plasma NE was 195 ± 53 pg/ml ($n = 4$) at base line. Intravenous administration of SKF 86466 (5 mg/kg) increased plasma NE by more than 20-fold (fig. 3). The maximum level of plasma NE (4275 ± 1295 pg/ml) was reached 60 min after the drug

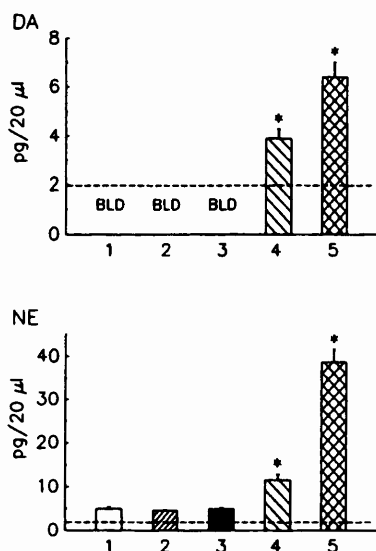

Fig. 1. Dose-response effect of SKF 86466 on dialysate DA and NE concentrations in the rat hippocampus. The values on the ordinate are the maximum concentrations of catecholamines, mean ± S.E.M. The number of animals in the base line (1) and saline (2) control groups was six, whereas the SKF 86466 (1 mg/kg) group (3) had 11 rats, SKF 86466 (5 mg/kg) group (4) had 14 rats and the SKF 86466 (10 mg/kg) group (5) had four rats. *Differs from base line, P < .05 (Student Newman-Keul's test); BLD, below the limit of detection. The broken line (------) indicates the limit of detection (2 pg/20 µl for DA and 1.5 pg/20 µl for NE). Dialysate DA was below the limit of detection (BLD) at base line (1), after saline administration (2) and after the 1 mg/kg dose of SKF 86466 (3).

injection. In contrast to the hippocampus where NE rapidly returned to base-line levels, the circulating levels of NE were elevated by more than 10-fold during the 240-min monitoring period. Plasma DA was not detected at base line or at any time after administration of SKF 86466.

Influence of the DA β-hydroxylase inhibitor. The possibility that DA was present in the hippocampal dialysate as a precursor of hippocampal NE was investigated with the DA β-hydroxylase inhibitor, SKF 102698. Intraperitoneal injection of 50 mg/kg of SKF 102698 was associated with a reduction in hippocampal dialysate NE below the limit of detection. DA was not detected in the hippocampal perfusate (fig. 4). SKF 86466 (5 mg/kg i.v.) administered 4 hr after the dopamine β-hydroxylase inhibitor increased dialysate DA in the 60-min fraction of the hippocampal perfusate but had no effect on dialysate NE (fig. 4).

Treatment with SKF 102698 effectively inhibited the DA β-hydroxylase, because plasma NE declined from the base-line concentration of 410 ± 40 pg/ml ($n = 6$) to 280 ± 80 pg/ml 4 hr after SKF 102698 administration. Plasma DA was increased from undetectable to 180 ± 60 pg/ml ($n = 6$; P < .05 compared to base line). When SKF 86466 was administered in two rats treated with the DA β-hydroxylase inhibitor, plasma DA was increased to 3140 and 3850 pg/ml, respectively, and plasma NE was increased to 4005 and 2950 pg/ml, respectively.

Local hippocampal infusion of inhibitors of DA or NE uptake. DA was not detected at base line or any of the samples from the control group that was infused with vehicle (artificial cerebrospinal fluid). Base-line levels for NE were 4.9 ± 1.1 pg/

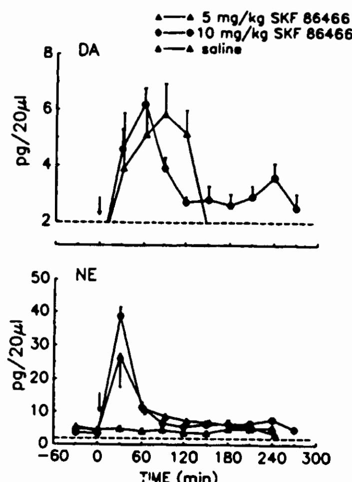

Fig. 2. DA and NE concentrations in the rat hippocampus at various times after administration of SKF 86466. The arrow denotes the intravenous injection of SKF 86466 or saline vehicle. The samples were collected at 30-min intervals. The ordinates give catecholamine concentrations as mean ± S.E.M. The number of animals in the saline control group and in the SKF 86466 (10 mg/kg) groups was four; in the SKF 86466 group it was three . The broken line (------) indicates the limit of detection (2 pg/20 µl for DA and 1.5 pg/20 µl for NE). DA was not detected at any time point after saline administration. For statistical analysis, the data points representing the samples below the limit of detection for DA were assigned an arbitrary value (1.99 pg/20 µl). The effects of both SKF 86466 doses were significantly different from saline (two-way MANOVA with repeated measures; time/treatment). DA 5 mg/kg dose, F = 7.46, P = .0002; 10 mg/kg, F = 8.09, P = .0001; NE, 5 mg/kg dose, F = 7.89, P = .0001; 10 mg/kg dose, F = 95.13, P < .00001.

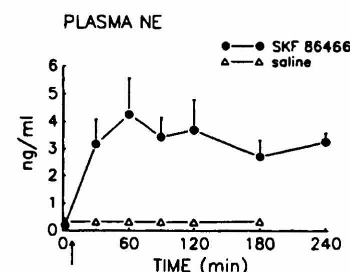

Fig. 3. Changes in plasma NE concentration after the intravenous injection of SKF 86466 (5 mg/kg). SKF 86466 or saline was injected at the time denoted by the arrow. The ordinate gives NE concentrations as mean ± S.E.M. The number of animals in the SKF 86466 group was four; in the saline group it was five. NE concentrations after SKF 86466 differed significantly from saline-treated controls (two-way MANOVA with repeated measures; time/treatment F = 4.90, P < .01).

20 µl, and were not affected by the vehicle infusion (fig. 5, C and D).

Hippocampal perfusion with 0.1 and 1 µM GBR-12909 dose-dependently increased DA levels (fig. 5A) up to 5.7 ± 1.2 (mean ± S.E.M.) and 9.6 ± 2.8 pg/20 µl, respectively. DA appeared in the first collection fraction during the drug perfusion and stayed at high levels during the 4-hr perfusion. When the drug was withdrawn, DA concentrations decreased dramatically, but remained detectable throughout the 90 min period after drug perfusion.

DA was also detectable during perfusion with 5 and 10 µM

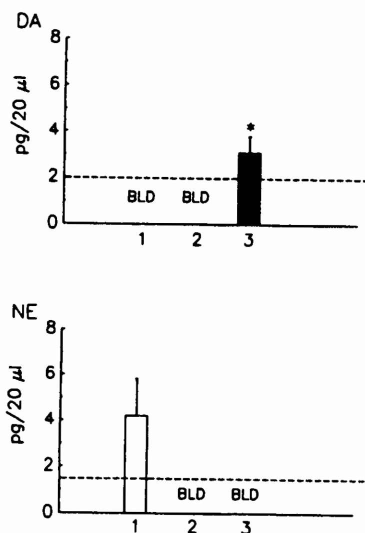

Fig. 4. Effect of SKF 86466 (5 mg/kg i.v.) on dialysate NE and DA concentrations in rats pretreated with the dopamine β-hydroxylase inhibitor, SKF 102698. SKF 102698 (50 mg/kg i.p.) was injected 4 hr before SKF 86466. (1) indicates baseline, (2) indicates treatment with the dopamine β-hydroxylase inhibitor, SKF 102698 and (3) indicates combined treatment with SKF 102698 and SKF 86466. Values are given as mean ± SEM. For measurement of dialysate DA (the upper panel) each group comprised five rats, for measurement of dialysate NE (lower panel) the base-line and SKF 102698 groups had eight rats whereas the third (SKF 102698 + SKF 86466) group had five rats. The broken line (——) indicates the limit of detection 2 pg/20 µl for DA and 1.5 pg/20 µl for NE. *Differs from baseline, $P < .05$ (Mann-Whitney U-test); BLD, below the limit of detection.

desipramine (fig. 5B). The maximum, 3.5 ± 0.6 pg/20 µl, was reached at the end of perfusion with 10 µM desipramine.

Desipramine increased NE levels in a dose-dependent manner (fig. 5D) to 8.7 ± 1.4 pg/20 µl (5 µM) and 14.1 ± 1.7 pg/20 µl (10 µM). There were no significant changes in NE levels after GBR-12909 (fig. 5C) nor in concentrations of DOPAC or HVA in any of the experimental groups (not shown).

Discussion

The basal extracellular concentration of NE (4.3 ± 0.3 pg/20 µl) in our study was comparable to findings in previous studies in which NE release in rat hippocampus was estimated by microdialysis (Abercrombie *et al.*, 1988; Kalén *et al.*, 1988; Abercrombie and Zigmond, 1989). The administration of SKF 86466, an *alpha*-2 adrenoceptor antagonist, was associated with an increase in dialysate NE in a dose-dependent manner with a rapid but relatively short-lasting time course. The increase in dialysate NE was not a reflection of changes in plasma NE because levels in the plasma were increased for more than 2-hr after SKF 86466 administration. The data are compatible with the hypothesis that presynaptic *alpha*-2 adrenoceptors regulate NE release in the hippocampus. The data are similar to findings after the administration of yohimbine, another *alpha*-2 adrenoceptor antagonist (Abercrombie *et al.*, 1988).

DA could not be detected in the base line or after administration of 1 mg/kg of SKF 86466, but it appeared in the dialysate after administration of SKF 86466 (5 and 10 mg/kg). To be certain that this peak represented DA, dialysate samples were analyzed by HPLC and the radioenzymatic assay in parallel with compatible results. Moreover, the retention time of the DA peak of the dialysis sample was identical with that of the standard DA peak when eluted with two different mobile phases. Therefore, the peak in the HPLC samples represents DA coming from the hippocampus. The possibility that circulating DA contributed to DA found in the hippocampal perfusate was excluded because DA was never above the limit of detection in the plasma samples.

The peak values of NE and DA after SKF 86466 treatment emerged at different dialysate fraction. The peak value of NE appeared at the first 30-min sample fraction and the levels returned back to base-line values within 90 min after the administration of SKF 86466. In contrast, DA in the dialysate reached maximum levels 60 min after administration of SKF 86466 and remained elevated throughout the collection period. The differential time-response of the increases in dialysate DA and NE may support the concept that DA and NE in the hippocampal dialysate have been released from separate terminals and thus DA detected in the hippocampal dialysate has not been derived from noradrenergic terminals but has been released from distinct dopaminergic neurons. However, the sustained release of DA could, at least in part, reflect overflow of DA which has been taken up from the extracellular space into the noradrenergic terminals as DA exhibits high affinity for the NE carrier (Raiteri *et al.*, 1977) and extracellular dopamine can be taken up into noradrenergic terminals (Carboni *et al.*, 1990).

The hippocampus is innervated by dopaminergic fibers (Bischoff *et al.*, 1979) originating from the ventral tegmental area and the substantia nigra (Scatton *et al.*, 1980; Swanson *et al.*, 1987). The presence of specific hippocampal DA receptors has been demonstrated by pharmacological, electrophysiological and radioligand binding techniques (Swanson *et al.*, 1987). However, there are only few DA neurons in the hippocampus and tissue levels of DA are low (Bischoff *et al.*, 1979; Verney *et al.*, 1985; Lategan *et al.*, 1992). Accordingly, DA was not detected in any of the base-line samples, which is in agreement with earlier studies (Abercrombie *et al.*, 1988; Bhardwaj *et al.*, 1990), but was only seen after treatment with the *alpha*-2 adrenoceptor antagonists or monoamine uptake inhibitors. In agreement with our data, DA was detected in the dialysates of gerbil hippocampus after induction of forebrain ischemia by ligation of the carotic arteries (Bhardwaj *et al.*, 1990). Because we verified the placement of the dialysis probe in the hippocampus in all animals, DA in dialysates most likely came from hippocampal sources. Therefore, it does not seem likely that diffusion from other brain regions would have significantly contributed to the DA detected in the hippocampal dialysates.

Several sites and mechanisms of action could account for the SKF 86466-induced DA release in hippocampus. Pharmacological studies have demonstrated the existence of *alpha*-2 adrenoceptor mediated inhibition of DA release in dopaminergic terminals in retinal neurons (Dubocovich, 1984) and in the hypothalamus (Ueda *et al.*, 1983). The *alpha*-2 agonist clonidine inhibited the evoked release of DA from rabbit retina in a dose-dependent manner whereas yohimbine, an *alpha*-2 adrenoceptor antagonist, completely antagonized the inhibitory effect of clonidine (Dubocovich, 1984). Administration of yohimbine, increased brain DA turnover (Papeschi and Theiss, 1975; Andén and Grabowska, 1976) presumably by an indirect action of yohimbine exerted through changes in NE neurotransmis-

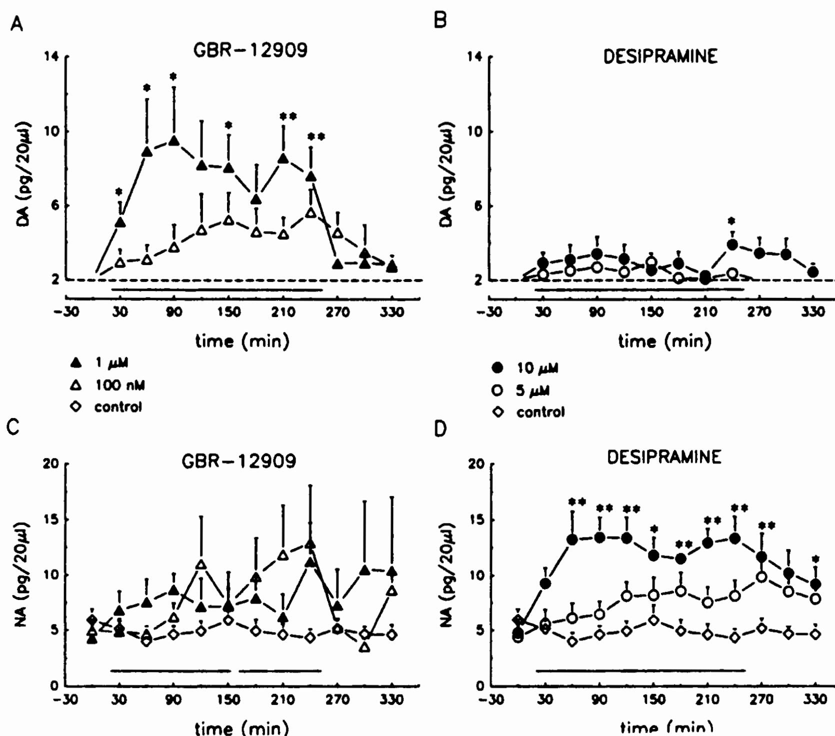

Fig. 5. Time course of GBR-12909 and desipramine on hippocampal monoamine levels in consecutive 30-min collection fractions. The effect of GBR-12909 on dialysate DA levels is depicted in A, and its effect on NE levels in C; open triangles, 0.1 µM of GBR-12909; closed triangles, 1 µM GBR-12909; open diamonds, control perfusion with artificial cerebrospinal fluid. The effect of desipramine on dialysate dopamine is denoted in B, and its effect on dialysate norepinephrine levels in D; open circles, 5 µM of desipramine; closed circles, 10 µM of desipramine; open diamonds, control perfusion with artificial cerebrospinal fluid. Values indicate mean ± S.E.M. Asterisks denote significance from control group; *$P < .05$, **$P < .01$ (Student Newman Keul's test). Dashed line indicates the limit of detection for dopamine (2.0 pg/20 µl) and the solid line denotes the drug perfusion period. Number of rats in each group was five, except for the GBR-12909 0.1 µM group it was four.

sion (Andén and Grabowska, 1976). We speculate that SKF 86466 increased DA release in the hippocampus results from a similar mechanism. Because SKF 86466 has shown to be a selective *alpha*-2 adrenoceptor antagonist (Hieble *et al.*, 1986; Daly *et al.*, 1988) with only weak activity at dopaminergic receptors (Dr. P. Hieble, personal communication), the possibility that these effects are mediated by blockade of presynaptic dopamine autoreceptors is unlikely. Furthermore, idazoxan, another selective *alpha*-2 antagonist, which has a very low affinity for DA receptors (Walter *et al.*, 1984), also increased hippocampal DA levels. Consequently, the detected DA release may be elicited by blockade of *alpha*-2 adrenoceptors.

The hippocampal DA might also derive from noradrenergic terminals where it serves as a precursor to NE synthesis (Brownstein *et al.*, 1974; Lindvall *et al.*, 1974; Westerink and Devries, 1985). If release from noradrenergic terminals had been the major source of DA in the present study, however, hippocampal release of DA should have been enhanced in rats treated with the DA β-hydroxylase inhibitor because this drug effectively inhibited the formation of NE in the noradrenergic neurons. On the contrary, our results demonstrated that the DA β-hydroxylase inhibitor did not affect basal hippocampal DA release. Furthermore, SKF 86466-induced release of hippocampal DA was not influenced by prior treatment with the DA β-hydroxylase inhibitor consistent with DA release from dopaminergic nerve terminals. In agreement with these data, it has been recently demonstrated that treatment of rats with the noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine resulted in a significant depletion of NE in the hippocampus without significantly affecting hippocampal DA levels or DA content of the caudate nucleus (Lategan *et al.*, 1992).

Further support for DA release from dopaminergic terminals in the hippocampus was furnished by the findings that local administration of GBR-12909, a selective DA uptake inhibitor, through the *in vivo* microdialysis tubing dose-dependently increased DA in hippocampal dialysates but had no significant effect on dialysate NE levels. Desipramine, a potent inhibitor of NE uptake and a weak inhibitor of DA uptake (Heikkila and Manzino, 1984; Shimada *et al.*, 1991), increased mainly dialysate NE concentrations. GBR-12909 is a potent and highly selective DA uptake inhibitor with no effect on NE uptake (Heikkila and Manzino, 1984; Andersen, 1989) and it has high affinity for a recently cloned dopamine transporter (Shimada *et al.*, 1991). In the striatum, locally applied GBR-12909 increased DA concentrations (Nomikos *et al.*, 1990). High concentrations of GBR-12909 were reported to directly release DA (Nomikos *et al.*, 1990). Doses of GBR-12909 used in our experiment are in a range where it acts as a pure DA uptake inhibitor and has no marked affinity for other known carriers or receptors (Andersen, 1989). Based on the selectivity of GBR-12909 for the DA transporter (Andersen, 1989; Shimada *et al.*, 1991) and the marked difference between the effects of GBR-12909 and desipramine on monoamine levels in the present experiment we conclude that the GBR-12909 induced increase in dialysate DA resulted from inhibition of DA transport sites in dopaminergic terminals.

The sharp onset and offset of the increase in dialysate DA during GBR-12909 infusion indicated that GBR-12909 probably acted on uptake sites different from the sites on noradrenergic terminals which were blocked by desipramine. The time course of the appearance of DA in the hippocampal dialysate as well as the magnitude of DA increase after desipramine were different from those elicited by infusion with the selective DA

uptake inhibitor. The gradual increase in dialysate DA during desipramine perfusion demonstrated, however, that the abundant noradrenergic terminals in the hippocampus could serve as an additional source for extracellular dopamine. During desipramine infusion, a profound dose-dependent increase in dialysate NE preceded the sustained increase in dialysate DA suggesting that the extracellular DA detected after desipramine infusion derived from noradrenergic terminals. This possibility is supported by the findings that DA exhibits high affinity for the NE carrier (Raiteri *et al.*, 1977) and that in NE-rich brain areas such as the prefrontal cortex, extracellular DA can be taken up into noradrenergic terminals (Carboni *et al.*, 1990). However, it could also be possible that desipramine at the relative high doses used in the present study could have inhibited DA uptake in dopaminergic terminals (Heikkila and Manzino, 1984; Shimada *et al.*, 1991).

In conclusion, the present study shows that 1) intracerebral microdialysis provides a useful method to monitor extracellular DA and NE levels in the hippocampal formation of the conscious rat; 2) administration of SKF 86466 increased the release of NE in hippocampal extracellular fluid emphasizing the fact that, in hippocampus, *alpha*-2 adrenoceptors exert important regulatory influences; 3) SKF 86466 increased the release of DA implying that *alpha*-2 adrenoceptors may also regulate DA release in the hippocampus and 4) selective blockade of the DA uptake process by GBR-12909 rapidly increased hippocampal DA levels, with no significant changes in the amounts of NE or secondary metabolites. Based on these findings, we propose that DA may be released directly from dopaminergic nerve terminals in the hippocampus.

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