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Long-term effects of an *Apocynum venetum* extract on brain monoamine levels and β-AR density in rats

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Abstract

The present study was designed to get further insight into the mode of antidepressant action of an extract prepared of the leaves of *Apocynum venetum* L. (AV). To evaluate biochemical changes, we used a high-performance liquid chromatography system to examine the effects of short-term (2 weeks) and long-term (8 weeks) administration of imipramine (15 mg/kg po) and an AV-extract (15, 60 and 250 mg/kg) on regional levels of serotonin (5-HT), norepinephrine (NE), dopamine (DA) and their metabolites in the rat hypothalamus, striatum and hippocampus. Pronounced changes in 5-HT, NE and DA levels were detected mainly after 8 weeks of daily imipramine treatment. Similar to imipramine, AV-extract reduced NE and DA concentrations after 8 weeks, whereas it failed to affect 5-HT levels. We speculate that the decrease in NE levels after chronic AV treatment might be based partly on the subsensitivity of presynaptic α_2-receptors. In addition to the determination of central monoamine concentrations, quantitative radioligand receptor-binding studies were used to examine the effects of long-term administration of imipramine and AV-extract on β-adrenergic binding in rat frontal cortex. [^{125}I]CYP binding to β-adrenergic receptors was found to be decreased after 8 weeks treatment with imipramine, whereas AV-extract had no effect on β-receptor binding.

Keywords: *Apocynum venetum*; Antidepressant; Serotonin; Dopamine; Norepinephrine; β-receptor regulation

1. Introduction

Apocynum venetum (AV) L. (Luobuma in Chinese, Apocynaceae) is a wild shrub widely distributed in mid and northwestern China. Since ancient times decoctions of AV leaves have been used in traditional Chinese medicine for the treatment of hypertension, nephritis and neurasthenia. Recently, teas prepared from AV leaves have become a popular healthy beverage in Japan. Previous papers describe the diuretic (Qing et al., 1988), antihypertensive, antihyperlipidaemic and anti-aging effects (Ma and Chen, 1989). Furthermore, aqueous extracts of AV leaves showed a cholesterol-lowering effect (Kim et al., 1998a), antilipid peroxidative activity (Yokozawa et al., 1997) and antioxidant effects on LDL from plasma of hypercholesterol-fed rats (Kim et al., 1998b). Recently, we have reported that an AV-extract markedly shortened the immobility time of male rats in the forced swimming test (FST) in a dose range of 30–125 mg/kg, indicating a possible antidepressant activity (Butterweck et al., 2001a). This effect was comparable to that of the tricyclic antidepressant imipramine (20 mg/kg). Neither imipramine (20 mg/kg) nor AV-extract in various doses (15, 60, 125 mg/kg) produced any overt behavioral change or motor dysfunction in the open-field test confirming the assumption that the antidepressant effect of an AV-extract in the FST is specific (Butterweck et al., 2001a).

In vivo studies examining the mechanism of antidepressant action of AV-extracts did not exist until now. Further, studies that investigate antidepressant effects after short-term (2 weeks) and long-term (8 weeks) administration of an AV-extract have not been conducted. Brady et al. (1991) were the first authors who used the 2 weeks/8 weeks treatment paradigm to examine the effects of various antidepressants on hypothalamic–pituitary–adrenal (HPA) axis activity. The authors found that CRH mRNA levels in the hypothalamic paraventricular nucleus

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(PVN) of rats were shown to be decreased following long-term (8 weeks) but not short-term (2 weeks) treatment with imipramine. Recently, we could replicate the imipramine- (Brady et al., 1991) and the fluoxetine-induced (Brady et al., 1992) delayed decreases in CRH mRNA levels in the PVN (Butterweck et al., 2001b). Based on the results, we speculate that a treatment period of longer than 14 days seems to be required for the detection of maximum biochemical effects. This hypothesis is further supported by our recent findings with St. John's wort and hypericin on changes in monoamine concentrations in different brain regions which were only observed after 8 weeks of daily treatment (Butterweck et al., 2002). Based on the results of Brady et al. (1991, 1992) and our own findings (Butterweck et al., 2001b), we used the 2 weeks/8 weeks treatment paradigm to investigate whether the effects on neurotransmitter concentrations and on β-AR-binding after 2 weeks differ or correlate with changes after 8 weeks.

Central norepinephrine (NE), serotonin (5-HT) and dopamine (DA) are suspected to play an important role in the etiology of depression. The extent of involvement of these neurotransmitters is not clearly understood nor is it known whether these amines are causally or secondarily related to the disorder (Delgado, 2000). Accordingly, the therapeutic effects of antidepressants are believed to be caused by central catecholaminergic or serotonergic systems. Thus, in the present study, we report on the effects of imipramine and AV-extract on rat brain levels of NE, DA and 5-HT and their metabolites after short- as well as long-term treatment.

A further common feature of many antidepressants is down-regulation (i.e. reduction in the number) of central β-adrenergic receptors (β-ARs) during chronic administration since this effect occurs about 2 to 3 weeks after initial administration of the drug (Vetulani et al., 1976; Sulser et al., 1978). This finding has provided a theory for the mechanism of action of antidepressants since several days of treatment are required for β-receptor subsensitivity to develop, which corresponds to the clinical effects of these drugs (Sulser et al., 1978). In the present paper, the action of AV-extract on the density of β-ARs was studied to gain further insight into the molecular mode of action of the plant extract. It was of further interest to find differences or similarities between the synthetic antidepressant imipramine and AV-extract in their ability to modify β-AR regulation after long-term administration.

2. Material and methods

2.1. Animals

Male CD rats (150–180 g, Charles River WIGA, Sulzfeld, Germany) were housed singly in a 12-h light/dark cycle, with lights off at 1800 h, at a constant temperature of 25 ± 1 °C and free access to food (Altromin 1324) and tap water. Rats were randomly assigned to the various experimental groups ($n = 12$/group) and weighed daily. The experimental procedures used in this work are in compliance with the European Communities Council Directive of 24th November 1986 (86/609/EEC) and were officially approved by the Regierungspräsident, Münster (A 92/99). Animals were sacrificed by decapitation between 0900 and 1100 h; the last drug administration was the day before between 1600 and 1700 h.

2.2. Substances and drug administration

Imipramine hydrochloride was obtained from Sigma (Deisenhofen, Germany). For all experiments, powdered *Apocynum* extract (drug/extract ratio: 25:2 prepared by Tokiwa Phytochemical, Chiba, Japan) was used.

Imipramine was dissolved in deionized water. *Apocynum* extract was dissolved homogeneously in water by sonication. Control animals received deionized water only. All substances were administered orally by gavage.

2.2.1. Preparation of Apocynum extract

Leaves of AV (100 g) were refluxed for 1 h in aqueous ethanol (70% v/v, 60 ml) two times and the combined alcoholic extractive was evaporated to dryness (28 g). The extract (13.5 g) was dissolved in hot water (200 ml), and adjusted to pH 3.0 with sulfuric acid, and then filtered. The filtrate was chromatographed on DAIAION HP-20 (3.6 cm id × 18 cm) and eluted with water (200 ml) and then aqueous ethanol (70% v/v, 200 ml). The aqueous ethanol fraction was collected and evaporated to dryness to obtain *Apocynum* extract (4.2 g). The extract was standardized on an amount of 2.1% hyperoside and 2.7% isoquercitrin, respectively. HPLC analytical conditions were as follows. Column: SHISEIDO CAPCELL PAKC18(UG) 46 mm id × 150 mm, detector at 330 nm, mobile phase: 0.1% TFA in water/0.1%TFA in acetonitrile 85:15, flow rate: 1.0 ml/min. Equipment: Waters 600 with waters 2487 UV detector. Hyperoside appeared at 6.5 min and isoquercitrin at 7.1 min. Authentic hyperoside and isoquercitrin were purchased from Funakoshi (Tokyo).

2.3. Dissections and tissue preparation

Brains were removed rapidly, dissected and the various regions weighed. Each dissected brain region was immediately homogenized (4 °C) with 1 ml of 0.2 M perchloric acid containing 0.05 M glutathione as antioxidant. The mixture was centrifuged at 2700 × *g* (4 °C) for 20 min, the pellet was discarded. The resultant supernatant was filtered (0.45 μm Nylon, Roth, Karlsruhe), and divided into two halves: 100 μl was directly injected into the HPLC for the determination of 5-HT, 5-HIAA, tyrosine and HVA, an aliquot of 900 μl was stored at − 80 °C for catecholamine analysis.

2.4. HPLC-ECD determination of indolamines and catecholamines and their major metabolites in different brain regions

The amounts of monoamines and their metabolites in various brain tissues were quantitatively measured by HPLC with ECD, details of the HPLC analysis and tissue preparations have been fully described by Butterweck et al. (2002). For quantification of the 5-HT, 5-HIAA, tyrosine and HVA, the electrochemical detector was coupled in line with a fluorescence detector (model F-1050, Merck; $\lambda_{ex} = 280$ nm; $\lambda_{em} = 315$ nm). HVA and 5-HIAA were detected amperometrically, tyrosine fluorometrically, and 5-HT was measured simultaneously on both detectors. Tissue levels were determined by means of external standards and expressed in terms of nanograms per gram of tissue. Correlation coefficients and calibration curves ($y = mx + b$) based on peak area ratios were established by means of linear regression analysis: 5-HT (ECD): 0.998, $55.9x - 4.27$; 5-HIAA (ECD): 0.997, $1.0511x - 0.07$; HVA (ECD): 0.998, $17.3x + 0.05$; tyrosine (fluorescence): 0.995, $1.81x + 0.07$; 5-HT (fluorescence): 0.999, $2.39x - 0.09$.

NE, DA, DOPA and DOPAC were detected electrochemically after solid-phase extraction on activated alumnia. Recoveries were approximately 75%. Tissue levels were determined by means of the internal standard dihydroxybenzylamine-hydrobromide (DHBA) and expressed in terms of nanograms per gram of tissue. Correlation coefficients and calibration curves ($y = mx + b$) based on peak area ratios were established by means of linear regression analysis: NE: 0.999, $19.3x - 0.1$; DA: 0.998, $22.7x - 0.21$; DOPA: 0.997, $15.36x + 0.24$; DOPAC: 0.999, $19.3x - 0.11$; DHBA (internal standard): 0.999, $26.4x + 0.39$.

2.5. Receptor-binding experiments

2.5.1. Membrane preparation

After decapitation, frontal cortices were dissected, immediately frozen in liquid nitrogen and stored at −80 °C until use. Brain tissue preparation was succeeded according to Koch et al. (1995) with small modifications. The tissue was homogenized in 10 mM HEPES buffer, containing 0.1 mM benzamidine and 10 mM EDTA (pH 7.4). The homogenate was centrifuged at $45{,}000 \times g$ for 15 min, the supernatant was discarded and the pellet was resuspended in the same buffer containing 1 mM EDTA and centrifuged two times under the same conditions. The final pellet was resuspended in 50 mM Tris–HCl containing 5 mM $MgCl_2$. The final tissue suspension was stored at −80 °C until use. Protein concentrations were determined according to Lowry et al. (1951).

2.5.2. Receptor-binding assays

To determine the affinity and density of the β-adrenergic receptors, radioligand receptor-binding assays were applied. As radioligand [^{125}I]cyanopindolol (ICYP), in a concentration range from 25 to 300 pM, was used. Protein and ICYP were incubated in 10 mM Tris–HCl, 154 mM NaCl, 0.1 mM ascorbic acid (pH 7.4) for 60 min at 37 °C. Nonspecific binding was determined by adding 20 μM alprenolol. The specificity for the β-adrenergic receptors was optimized by excluding ICYP binding to 5-HT_{1B}-receptors with 10 μM 5-HT (Hoyer et al., 1985). After incubation the reaction was stopped by the addition of ice-cold 0.9% NaCl, samples were rapidly filtered using Whatman GF/B filters and a Brandel Cell Harvester, and bound and free ligands were separated by washing twice with ice-cold 0.9% NaCl. Filters were counted in a Cobra II auto-gamma counter at 80% counting efficiency (Canberra-Packard, Dreieich). ICYP was purchased from NEN Life Sciences Products (Cologne, Germany), alprenolol-HCl and 5-HT were from Sigma (Taufkirchen, Germany).

2.6. Data analysis and statistics

Radioligand binding data were analyzed by a nonlinear least square method using GRAPHPAD Prism. All statistical procedures were performed by use of the STATVIEW statistical software package, version 5.0 (SAS, USA). Data analysis was performed by analysis of variance (ANOVA) with the Student–Newman–Keuls post hoc test for multiple comparisons. Data are expressed as means ± S.E.M. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Effects of imipramine and AV-extract on tyrosine, DA, NE and their metabolites in rat brain tissues

The content of catecholamines in the hypothalamus, striatum and hippocampus after short-term (2 weeks) and long-term (8 weeks) treatment is shown in Table 1. The basal levels of catecholamines and their metabolites are in line with data from literature (Mousseau and Greenshaw, 1989; Chung et al., 1993; Butterweck et al., 2002). Daily treatment with imipramine (15 mg/kg po) or AV-extract (15, 60 and 250 mg/kg) produced no significant alterations in tyrosine levels in either of the investigated brain regions. Comparable to imipramine, NE levels were significantly reduced in the hypothalamus and striatum after 8 weeks of daily treatment with 15 and 60 mg/kg of the AV-extract ($P < 0.05$). In the striatum and hippocampus, the decrease of NE levels occurred already after 2 weeks of daily treatment with imipramine and the AV-extract. Whereas all three doses of the AV-extract significantly reduced NE levels ($P < 0.05$) after short-term treatment in the striatum, only 250 mg/kg produced a similar effect in the hippocampus.

Table 1
Effects of short-term and long-term administration of imipramine (15 mg/kg), AV-extract (15, 60 and 250 mg/kg) on tyrosine and its metabolites in various regions of the rat brain

Brain region	Control		Imipramine (15 mg/kg)		Apocynum (15 mg/kg)		Apocynum (60 mg/kg)		Apocynum (250 mg/kg)	
	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks
Hypothalamus										
Tyrosine (ng/g)	1432 ± 80	1367 ± 39	1450 ± 57	1404 ± 58	1416 ± 26	1251 ± 66	1426 ± 34	1435 ± 66	1356 ± 84	1228 ± 70
DOPA (ng/g)	n.d.	39 ± 10	n.d.	25 ± 4	n.d.	30 ± 4	n.d.	28 ± 4	n.d.	30 ± 5
DA (ng/g)	237 ± 23	314 ± 30	274 ± 17	240 ± 28 *	261 ± 14	209 ± 15 * *	253 ± 15	317 ± 16	247 ± 12	306 ± 24
NE (ng/g)	1314 ± 22	1636 ± 51	1148 ± 100	936 ± 69 * *	1268 ± 85	919 ± 66 * *	1320 ± 36	1147 ± 25 * *	1424 ± 68	1504 ± 68
DOPAC (ng/g)	26 ± 7	63 ± 7	34 ± 4	47 ± 5	36 ± 9	50 ± 4	29 ± 8	60 ± 8	27 ± 6	60 ± 6
HVA (ng/g)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Striatum										
Tyrosine (ng/g)	1302 ± 60	1255 ± 53	1342 ± 24	1259 ± 65	1355 ± 36	1185 ± 52	1334 ± 39	1248 ± 73	1393 ± 49	1124 ± 36
DOPA (ng/g)	21 ± 7	35 ± 4	24 ± 4	42 ± 8	19 ± 2	35 ± 5	20 ± 5	27 ± 3	25 ± 4	31 ± 7
DA (ng/g)	1673 ± 137	1694 ± 32	1519 ± 112	1608 ± 47	1952 ± 189	1590 ± 48	1773 ± 142	1677 ± 40	1755 ± 121	1601 ± 67
NE (ng/g)	201 ± 25	173 ± 20	192 ± 24	131 ± 10 *	136 ± 16 *	107 ± 13 *	132 ± 12 *	135 ± 9 *	124 ± 16 *	200 ± 23
DOPAC (ng/g)	827 ± 26	1025 ± 41	904 ± 71	698 ± 63 * *	857 ± 60	808 ± 70 *	839 ± 29	535 ± 62 * *	869 ± 57	771 ± 87 *
HVA (ng/g)	521 ± 23	564 ± 29	427 ± 24	540 ± 22	519 ± 19	512 ± 22	546 ± 20	526 ± 25	597 ± 80	544 ± 39
Hippocampus										
Tyrosine (ng/g)	1221 ± 46	1218 ± 35	1211 ± 30	1199 ± 67	1296 ± 46	1083 ± 50	1245 ± 39	1218 ± 52	1195 ± 39	1095 ± 36
DOPA (ng/g)	n.d.	15 ± 3	n.d.	14 ± 2	n.d.	33 ± 11	n.d.	21 ± 4	n.d.	18 ± 5
DA (ng/g)	n.d.	21 ± 3	n.d.	23 ± 4	n.d.	18 ± 6	n.d.	15 ± 3	n.d.	14 ± 3
NE (ng/g)	312 ± 14	310 ± 34	262 ± 11 *	346 ± 52	287 ± 10	236 ± 48	284 ± 13	348 ± 26	275 ± 7 *	243 ± 31
DOPAC (ng/g)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
HVA (ng/g)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Values are mean ± S.E.M. expressed as ng/g tissue of 12 animals in each group. Data analysis was performed by analysis of variance with Student–Newman–Keuls post hoc test for multiple comparisons.
ND = not detectable; DOPA = 3,4-dihydroxyphenylalanine; DA = dopamine; NE = norepinephrine; DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid.
* $P < 0.05$.
** $P < 0.01$.

In the hypothalamus, imipramine, as well as 15 mg/kg of the AV-extract, reduced levels of DA ($P < 0.05$) but only after 8 weeks of daily treatment. Pronounced changes in DOPAC concentrations were observed in the striatum: imipramine as well as AV-extract (15, 60 and 250 mg/kg) markedly decreased DOPAC levels after long-term treatment.

Table 2
Effects of short-term (2 weeks) and long-term (8 weeks) administration of imipramine (15 mg/kg), AV-extract (15, 50 and 250 mg/kg) on 5-HT and 5-HIAA levels in the hypothalamus, striatum and hippocampus of the rat brain

Brain region	Control		Imipramine (15 mg/kg)		Apocynum (15 mg/kg)		Apocynum (60 mg/kg)		Apocynum (250 mg/kg)	
	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks	2 weeks	8 weeks
Hypothalamus										
Tryptophane (ng/g)	272 ± 11	269 ± 9	255 ± 17	274 ± 8	256 ± 7	273 ± 12	261 ± 11	261 ± 16	255 ± 15	281 ± 13
5-HT (ng/g)	422 ± 28	405 ± 22	437 ± 12	515 ± 17 * *	403 ± 9	477 ± 21	404 ± 28	492 ± 21	394 ± 23	462 ± 14
5-HIAA (ng/g)	95 ± 5	111 ± 8	96 ± 5	112 ± 8	93 ± 5	96 ± 7	98 ± 7	113 ± 8	103 ± 8	116 ± 8
Striatum										
Tryptophane (ng/g)	257 ± 11	234 ± 8	246 ± 8	248 ± 10	240 ± 7	248 ± 6	240 ± 8	243 ± 15	238 ± 10	239 ± 10
5-HT (ng/g)	265 ± 7	280 ± 12	279 ± 13	286 ± 13	277 ± 12	309 ± 13	290 ± 12	312 ± 15	240 ± 9	270 ± 10
5-HIAA (ng/g)	70 ± 4	72 ± 3	72 ± 4	92 ± 6 * *	75 ± 6	79 ± 3	73 ± 3	86 ± 6	66 ± 10	76 ± 5
Hippocampus										
Tryptophane (ng/g)	244 ± 12	200 ± 9	246 ± 9	199 ± 10	224 ± 5	203 ± 11	253 ± 6	212 ± 10	227 ± 13	208 ± 7
5-HT (ng/g)	169 ± 5	163 ± 11	168 ± 6	194 ± 9	161 ± 9	194 ± 7	170 ± 9	184 ± 7	167 ± 8	174 ± 10
5-HIAA (ng/g)	57 ± 5	72 ± 3	56 ± 2	93 ± 7 *	50 ± 3	76 ± 3	58 ± 4	92 ± 9	50 ± 4	71 ± 7

Values are mean ± S.E.M. expressed as ng/g tissue of 12 animals in each group. Data analysis was performed by analysis of variance with Student–Newman–Keuls post hoc test for multiple comparisons.
5-HT = 5-hydroxytryptamine; 5-HIAA = 5-hydroxyindoleacetic acid.
* $P < 0.05$.
** $P < 0.01$.

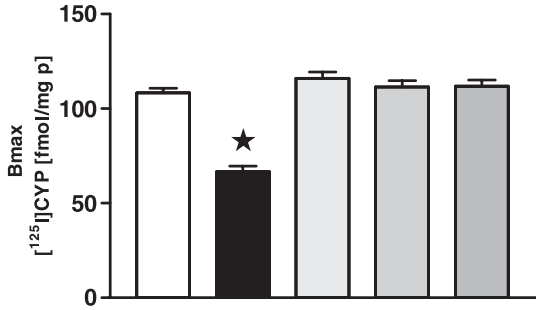

Fig. 1. Effects of long -term treatment with imipramine (15 mg/kg) and AV-extract (15, 60 and 250 mg/kg) on density of β-adrenergic receptors in rat frontal cortex (□ control; ■ imipramine 15 mg/kg; AV-extract 15 mg/kg; ▒ AV-extract 60 mg/kg; ▓ AV-extract 250 mg/kg). Data are expressed as mean ± S.E.M. of 8–12 individual animals. Statistical differences were determined using ANOVA followed by Student–Newman–Keuls post hoc test. * $P<0.05$, ** $P<0.01$.

3.2. Effects of imipramine and AV-extract on 5-HT and 5-HIAA levels and turnover in rat brain tissues

The content of indoles in the hypothalamus, striatum and hippocampus after short-term (2 weeks) and long-term (8 weeks) treatment is shown in Table 2. The basal levels of 5-HT and its metabolites are in line with data from literature (Chung et al., 1993).

In all three brain regions, only imipramine (15 mg/kg) affected 5-HT metabolism. The AV-extract had no effect on brain indolamine concentrations. In the hypothalamus, imipramine, administered for 8 weeks, but not for 2 weeks, significantly increased 5-HT levels ($P<0.05$). 5-HIAA levels were significantly increased after long-term treatment with imipramine in the striatum ($P<0.01$) and hippocampus ($P<0.05$).

3.3. Effects of long-term treatment with imipramine and AV-extract on density of β-adrenergic receptors in rat frontal cortex

Fig. 1 shows the effect of treatment with imipramine (15 mg/kg) and AV-extract (15, 60 and 250 mg/kg) on ^{125}ICYP-binding to β-adrenergic receptors in rat frontal cortices after long-term administration. Imipramine (15 mg/kg) induced a significant decrease (39%) in the number of β-adrenergic receptors in the frontal cortex (B_{max}). Long-term treatment with an AV-extract caused no significant alteration of ICYP binding to β-ARs.

4. Discussion

Data from clinical studies provide evidence that it takes 2 to 3 weeks for antidepressant effects to become evident (Montgomery, 1995; Katz et al., 1996/1997), and that maximal therapeutic effects of antidepressants occur after about 8–12 weeks (Quitkin et al., 1984, 1991, 1996). In the present study, we analyzed the effects of the tricyclic antidepressant, imipramine, and an extract prepared from the leaves of AV on brain monoamine concentrations and β-AR binding after short-term (2 weeks) and long-term drug administration (8 weeks). It was one aim of this study to investigate whether the effects on monoamine levels and β-AR binding after 2 weeks differ or correlate with changes after 8 weeks.

The major novel finding of this study is that both imipramine and AV-extract affected concentrations of monoamine neurotransmitters and their metabolites mainly after long-term administration. The concentration of NE was not changed after short-term (2 weeks) treatment with either imipramine or AV-extract in the hypothalamus, but it was reduced in the striatum and hippocampus after 2 weeks of daily administration of both drugs. The decrease in NE levels was still obvious after 8 weeks for both drugs in the hypothalamus and striatum. Interestingly, only 250 mg/kg of the AV-extract reduced levels of NE in the hippocampus after 2 weeks, whereas in the hypothalamus and the striatum, lower doses of the extract (15 and 60 mg/kg) were necessary to produce a similar decrease of NE levels. The decreased NE levels observed after long-term imipramine treatment are in good correlation with our previous results (Butterweck et al., 2002). Interestingly, an extract of AV produced similar changes in central NE concentrations as the tricyclic antidepressant suggesting a similar mode of action. A decrease in NE levels in the whole rat brain was also observed by Sedlock and Edwards (1985) and Sugita et al. (1987). After 2 weeks of treatment with desipramine, the concentration of NE decreased in the hippocampus and thalamus, and increased in the corpus striatum (Chung et al., 1993). Karoum et al. (1984) reported a decreased NE turnover in the hypothalamus after chronic treatment with desipramine. It was reported recently that clonidine, infused into the dorsal hippocampus through a microdialysis probe, significantly lowered extracellular NE, to a similar extent in rats chronically treated with desipramine for 14 days (Sacchetti et al., 2000). The authors suggest that these changes in the brain might be based partly on the subsensitivity of presynaptic α_2-receptors (Sacchetti et al., 2000). Presynaptic α_2-receptors control the release of NE from central neurons (Langer, 1981). Activation of the α_2-receptors by clonidine decreases NE release (Svensson and Usdin, 1978; Sacchetti et al., 2000). Studies in rats indicate that chronic, but not acute, treatment with desipramine results in a decreased sensitivity of α_2-receptors and an alteration in the synthesis and release of NE from the neuron (Spyraki and Fibiger, 1980). Thus, it is possible that the decreased concentration of hypothalamic, striatal and hippocampal NE observed in the present study after repeated treatment with imipramine or AV-extract resulted from an enhanced metabolism and/or from the augmented depletion of the stored NE by the decreased density and sensitivity of the presynaptic α_2-receptors which inhibit NE release upon stimulation. This

interesting possibility remains to be explored by further experimentation.

In the present study, a decreased concentration of striatal DOPAC was observed after long-term treatment with imipramine (15 mg/kg) and the AV-extract in concentrations of 15 and 60 mg/kg, respectively. At the same time, imipramine and 15 mg/kg of the AV-extract reduced DA levels in the hypothalamus. We have previously reported that long-term administration of imipramine (15 mg/kg po), a methanolic St. John's wort extract (500 mg/kg po), or hypericin (0.2 mg/kg po) all decreased DOPAC levels in rat brain tissues (Butterweck et al., 2002), showing parallels between both plant extracts. Our present observations on reduced DOPAC levels are further in good correlation with previous findings from other working groups (Karoum et al., 1984; Mousseau and Greenshaw, 1989; Chung et al., 1993). Serra et al. (1979, 1980) proposed the involvement of dopaminergic presynaptic receptors in the action of antidepressants. They observed that chronic treatment with tricyclic antidepressants reduced the effect of a small dose of apomorphine by stimulating dopaminergic presynaptic receptors. Moreover, it has been reported that amineptine, which blocks DA reuptake, had antidepressive activity (Simoni et al., 1986) and that tricyclic antidepressants themselves could decrease the concentration of DOPAC (Holcomb et al., 1982; Digory and Buckett, 1984). In the present study, a decreased concentration of striatal DOPAC was observed after long-term treatment with the tricyclic antidepressant imipramine or an AV-extract (15 and 60 mg/kg). It can be speculated that a blockade of DA reuptake may be responsible for the reduced concentration of DOPAC since there was no alterations in the concentrations of HVA, an extracellular metabolite, after both treatments.

Alterations of the concentrations of 5-HT and/or 5-HIAA were found in all three investigated brain regions but only after long-term imipramine treatment. Imipramine markedly increased 5-HT levels in the hypothalamus. 5-HIAA levels were increased in the striatum and hippocampus in a similar quantitative manner. Interestingly, AV-extract had no effect on 5-HT and/or 5-HIAA in all three different doses investigated. Increased levels of hypothalamic 5-HT levels after long-term imipramine treatment have been reported in our recent paper (Butterweck et al., 2002). An increase in 5-HT extracellular levels in the hippocampus and striatum was also detected in rats treated with fluoxetine for 14 days, 48 h after the last administration (Kreiss and Lucki, 1995) and in the frontal cortex, 12 h after the last injection in rats treated for 14 days with citalopram (Arborelius et al., 1996). Furthermore, in homogenates of brain regions, repeated tianeptine treatment increased 5-HT and 5-HIAA levels in the hypothalamus and hippocampus. The present work demonstrated that imipramine—in contrast to AV-extract—increases brain regional levels of 5-HT and its metabolite 5-HIAA. This increase may trigger the chain of events which lead to the therapeutic effects of this compound.

A further interesting result of our present study is that long-term treatment with imipramine led to a significant down-regulation of β-adrenergic receptors, whereas long-term treatment with AV-extract had no effect on β-adrenergic densities in none of the tested doses. Based on the former hypothesis that down-regulation of β-adrenergic receptors is a common biochemical marker of antidepressant activity (Vetulani et al., 1976; Sulser et al., 1978), the lack of effect of AV-extract on β-adrenergic receptor regulation could be interpreted as inactivity of the plant extract.

However, the majority of studies with the selective serotonin reuptake inhibitor (SSRI) fluoxetine, as well as recent studies with venlafaxine, a dual amine reuptake inhibitor, indicated that β-adrenoceptor down-regulation is not necessarily a prerequisite for antidepressant activity (Mishra et al., 1979; Maggi et al., 1980; Peroutka and Snyder, 1980; Fuxe et al., 1983; Stolz et al., 1983; Wong et al., 1985; Baron et al., 1988; Nalepa et al., 1998). Moreover, the studies of venlafaxine mandated a further shift of the mode of action of antidepressants from the receptor level to 5-HT and NE receptor-coupled intracellular transduction (Nalepa et al., 1998). Recent in vivo studies established that, notwithstanding β-AR down-regulation, long-term antidepressant treatments lead to sustained activation of the cyclic AMP system in specific brain regions that leads to activation of cyclic AMP response element-binding protein (CREB). CREB could be regulated by monoamine receptors that couple to the cAMP-PKA cascade (5-$HT_{4,6,7}$ and β-AR) (Duman et al., 1997a,b). However, the cAMP cascade is just one of many intracellular pathways that could be regulated by 5-HT and NE and could be important to the action of antidepressant treatments. Thus, to get a further insight into the mode of antidepressant action of AV-extract, further investigations on receptor-coupled intracellular pathways are of interest.

In conclusion, the results of our present study show that long-term treatment with an extract of AV induced several changes in the NE system, which were comparable to those of imipramine. In contrast to imipramine, which also altered 5-HT levels in several brain regions, AV-extract selectively affected the noradrenergic system. Thus, we speculate that these changes in the brain might be based partly on the subsensitivity of presynaptic α_2-receptors. Taken together, our results clearly show that there are similarities and differences between the tricyclic antidepressant imipramine and the herbiceutical AV in their ability to modify brain monoamine neurotransmission in the rat. Further in vivo studies are needed to establish the pharmacological relevance of these findings for therapeutic usage of AV.

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