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Decreased Hypothalamic Serotonin Levels in Adult Rats Treated Neonatally with Clomipramine

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FEENSTRA, M. G. P., H. VAN GALEN, P. J. M. TE RIELE, M. H. A. BOTTERBLOM, M. MIRMIRAN. *Decreased hypothalamic serotonin levels in adult rats treated neonatally with clomipramine.* PHARMACOL BIOCHEM BEHAV **55**(4) 647–652, 1996.— Early postnatal treatment with the antidepressant drug clomipramine has repeatedly been shown to lead to behavioural and physiological changes in adult rats. To provide some neurochemical correlates to these studies we have measured a number of monoaminergic parameters in the brains of adult (one year old) rats that were treated twice daily with 15 mg/kg clomipramine from postnatal day 2–14. The most consistent finding was that the hypothalamic levels of serotonin (5-HT) were decreased and those of the dopamine metabolite dihydroxyphenylacetic acid (DOPAC) were increased in rats irrespectively whether they went through a range of behavioural and physiological tests or not. The numbers of β-adrenoceptors in the frontal cortex and of α_2-adrenoceptors in the amygdala/piriform cortex were not changed. The decrease in hypothalamic 5-HT concentrations appears to be up to now the most consistent neurochemical alteration in adult rats that were neonatally treated with antidepressant drugs. It is, however, not clear what the relation is with the functional changes in these rats, that are proposed by some authors as an animal model for depression.

Clomipramine Serotonin Dopamine metabolism Hypothalamus Antidepressant drugs Adrenergic receptors Depression Brain development

TREATMENT with antidepressant drugs during the first weeks of postnatal life of rats has been shown to lead to long-lasting alterations in physiological and behavioural parameters. These include increased ambulation and exploration of an open field, increased percentage of active or REM sleep, altered circadian rhythmicity, decreased male sexual performance, increased voluntary intake of ethanol, increased immobility in a forced swim test, decreased intracranial self stimulation and decreased shock-induced aggression, in the absence of clear changes in learning and memory functions (3,12,13,14,15,20,21,23,24,26, 27,28; for negative findings see 9). The observed changes have been suggested to model endogenous depression (12,28). Many authors suggested that long-lasting alterations in neurotransmitter functioning would underlie these changes. Yet, a very limited number of neurochemical measurements in adult rats that were neonatally exposed to antidepressants have been reported, while behaviour was comprehensively studied. In the present study rats were treated with the tricyclic antidepressant clomipramine during the first two weeks of postnatal life. Clomipramine and its demethylated metabolite are potent inhibitors of serotonin (5-HT) and noradrenaline (NA) reuptake, respectively (16). As NA and 5-HT pathways appear early in development and high affinity uptake mechanisms are functional already before birth, clomipramine can be expected to be pharmacologically active during this early postnatal treatment (2,17,25). We determined monoamines and metabolites as general indicators of regional monoaminergic systems and α_2- and β-adrenoceptors that are subject to profound changes directly after chronic antidepressant treatments (11). The measurements were carried out in brain tissue of adult, i.e. one year old rats.

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METHODS

Animals and Treatment

Offspring from Wistar rats mated in our institute were born on day 21 of pregnancy (which equals postnatal day 0) and six nests were made consisting of six males and one female. All pups of three nests were injected between postnatal days 2 and 14 twice daily subcutaneously with saline and all pups from three other nests with 15 mg/kg clomipramine (3). After weaning at PN 22 the males were housed in groups of four. The rats were divided into two groups. The rats in the first group (A) were used from PN 84-175 in maze tests, from PN 231-246 male sexual behaviour was evaluated and from PN 280-350 sleep measurements were carried out (3). This group consicted of 7 males from the saline-treated nests and 8 from the clomipramine-treated nests. The second group (B) consisted of 9 controls and 10 clomipramine-exposed. These went through the same schemes of weighting and food deprivation but were not used in the behavioural tests listed above. They did not undergo surgery for the placement of cranial electrodes and were not used for sleep measurements. When the rats were one year old they were decapitated in random order in separate sessions for groups A and B and their brains were rapidly dissected (8). Four regions were immediately frozen on dry ice and stored at −80°C until analysis. The mean weights of these regions of the control (groups A + B) animals were (mg ± SEM, *n*): Hypothalamus 44.9 ± 1.7 (16); Medulla-Pons 253.6 ± 4.7 (13); Frontal Cortex 127.7 ± 4.0 (16); Amygdala/Piriform Cortex 115.9 ± 4.7 (16).

All tissue pieces obtained from experiment A were used for measurements of monoamine metabolism using HPLC with amperometric detection. The frontal cortex and amygdala/piriform cortex pieces from group B were used exclusively for measurement of adrenergic receptors. The amount of tissue needed for these measurements precluded the use of these tissues for HPLC determinations. The hypothalamus and medulla-pons pieces from group B were used for HPLC determinations.

HPLC Analysis

Frozen pieces of hypothalamus or amygdala/piriform cortex tissue were homogenized in 1.0 ml 0.1 M perchloric acid (PCA), containing 0.3 mM EDTA and 0.01 mM ascorbic acid. Frontal cortex was homogenized in 1.5 ml and medulla-pons in 2.0 ml. Homogenization and further processing was done batch-wise separately for each region from each experiment. After centrifugation for 15 min at 3000 g 1.0 ml supernatant was transferred to small Sephadex G-10 columns (29). This prepurification was carried out to allow the quantification of the NA metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) in its free, unconjugated form. After a 1.0 ml wash with 0.01 M formic acid three fractions were eluted using a modification of the original scheme of Westerink (29): after application of 1.0 ml 0.01 M formic acid 1.0 ml eluate was collected in which MHPG was present with a recovery of 50–60%. A second fraction of 2.0 ml eluate was collected after application of 1.5 ml 0.01 M formic acid and 0.5 ml 0.005 M Na_2HPO_4, in which NA and dopamine (DA) were present with recoveries of 80–90%. The third fraction was eluted after application of 2.0 ml 0.1 M KOH and collected in tubes with 25 µl formic acid and 25 µl 0.4 mM ascorbic acid. 5- HT, 5-HIAA, DOPAC and homovanillic acid (HVA) were present in this eluate with recoveries of 60–80%. A volume of 250 µl of all fractions was injected either in a HPLC system consisting of a Waters WISP 712 autosampler (cooled to 4°C), a Waters 600E solvent delivery system in connection with a Supelcosil 5C18DB reversed phase column (25 × 0.46 cm) and precolumn (2 × 0.46 cm), both kept at 40°C, and a Waters 460 amperometric detector operated at 800 mV against a Ag/AgCl reference electrode or in a system consisting of a Hewlett Packard 1090 liquid chromatograph with autosampler, connected to similar Supelcosil columns kept at 40°C, and a Metrohm 641 VA amperometric detector operated at 800 mV. The eluent for the MHPG fraction was a citrate/acetate buffer (pH 4.2) with 0.3 mM EDTA and 8% methanol, the eluent for the amine fraction was the same but with 0.75 mM heptanesulphonic acid (HSA) added, and the eluent for the third fraction contained 15% methanol and 0.85 mM HSA. Peak heights were compared with external standards. Recoveries were determined by addition of known amounts of the monoamines and metabolites to cerebellar tissue pieces.

Receptor Assays

Receptor measurements in all individual tissue pieces (frontal cortex for β-adrenoceptors and amygdala/piriform cortex for α_2-adrenoceptors) were carried out at a fixed ligand concentration. In addition, saturation curves with 6–10 ligand concentrations were determined in tissue fractions from two rats. In this way maximal information was obtained from the limited amount of tissue available. Thus, no tissue was left for HPLC determinations.

β-Adrenergic receptors were measured according to Erdtsieck-Ernste et al (6) using [^{125}I]iodocyanopindolol. All individual tissue pieces (frontal cortex) were homogenized (25 mg/ml) and aliquots were used for a binding assay using a ligand concentration of 195 pmol/l. Further aliquots from two different rats from the same treatment group were combined for a saturation binding curve with 10 ligand concentrations from 6.25 to 200 pmol/l. Crude membranes were obtained after centrifugation at 500g. About 0.2 g/l protein was incubated with the ligand for 30 min at 37°C in a 50 mmol/l Tris buffer with 5 mmol/l $MgCl_2$, 145 mmol/l NaCl and 0.6–6 µmol/l 5-HT. Aspecific binding was determined by addition of 5 µmol/l timolol. Binding to β_2-receptors was determined by addition of the selective β_1-antagonist ICI 89406 (7–200 nmol/l). 5-HT and ICI 89406 concentrations depend on the ligand concentration (6).

α_2-Adrenoceptors were measured according to Boer et al (1). All individual tissue pieces (amygdala/piriform cortex) were homogenized (20 mg/ml) and aliquots were used for a binding assay using a ligand concentration of 10 nmol/l. Further aliquots of tissue pieces from two different rats from the same treatment group were combined for a saturation binding curve with 6 ligand concentrations from 0.3 to 10 nmol/l. Crude, washed membranes were obtained after centrifugation at 48000 g. About 0.2 g protein/l was incubated with the ligand for 30 min at 25°C in a 50 mmol/l Tris/HCl buffer with 0.5 mmol/l $MgCl_2$. Aspecific binding was determined by addition of 10 µmol/l phentolamine. Binding data were analyzed using EBDA/LIGAND (Elsevier/Biosoft see McPherson (19).

Statistical Analysis

Evaluation of the results of the HPLC determinations in hypothalamus and medulla-pons was performed by two-way analysis of variance, with main effects for group (A or B) and neonatal treatment (saline or clomipramine). All other results were tested with oneway analysis of variance. Post-hoc com-

TABLE 1
MONOAMINES AND METABOLITES

	Group A		Group B	
	Control	Clomipramine	Control	Clomipramine
Hypothalamus				
NA	1696 ± 145	1589 ± 177	1279 ± 39	1370 ± 31
DA	340 ± 110	295 ± 50	293 ± 12	285 ± 44
DOPAC	39.0 ± 3.6	48.0 ± 3.5*	27.9 ± 1.9	34.1 ± 0.8*
HVA	12.0 ± 3.3	16.4 ± 1.6	24.0 ± 1.5	19.0 ± 2.1
5-HT	1126 ± 96	894 ± 36*	1031 ± 35	826 ± 25*
5-HIAA	719 ± 27	668 ± 70	606 ± 23	488 ± 20*
Medulla-pons				
NA	422 ± 15	417 ± 29	399 ± 8	407 ± 10
MHPG	ND	ND	57.9 ± 1.8	61.4 ± 0.7
DA	39.3 ± 0.6	41.8 ± 1.9	41.7 ± 1.3	42.3 ± 0.5
DOPAC	15.8 ± 1.9	20.5 ± 1.4*	15.8 ± 0.9	15.7 ± 0.5
HVA	10.8 ± 1.9	12.2 ± 1.8	15.9 ± 1.7	14.2 ± 1.3
5-HT	462 ± 21	465 ± 13	481 ± 10	456 ± 14
5-HIAA	378 ± 8	384 ± 30	422 ± 16	377 ± 18
Frontal cortex				
NA	490 ± 20	517 ± 16		
MHPG	13.6 ± 1.3	15.9 ± 2.8		
DA	55.1 ± 4.2	54.5 ± 2.4		
DOPAC	19.3 ± 2.3	18.8 ± 1.4		
HVA	22.9 ± 2.8	26.9 ± 3.9		
5-HT	584 ± 16	611 ± 19		
5-HIAA	283 ± 8	306 ± 18		
Amygdala/piriform cortex				
NA	484 ± 16	508 ± 21		
MHPG	5.3 ± 0.5	6.0 ± 0.5		
DA	482 ± 71	510 ± 70		
DOPAC	47.1 ± 6.2	48.3 ± 6.3		
HVA	27.6 ± 5.4	29.7 ± 5.8		
5-HT	694 ± 24	732 ± 20		
5-HIAA	309 ± 9	333 ± 17		

All values are means ± SEM expressed in ng/g wet weight. The number of observations in goup A was 6–8 (only in medulla-pons 4–6) and in group B 9—10.
*Significantly different from control values in the same experiment.

parisons were assessed with the Student-Newman-Keuls t-test. In all tests $\alpha = 0.05$ was used.

RESULTS

Monoamine Metabolism

The results of the HPLC determinations are shown in Table 1. The individual metabolite/transmitter ratio's were also calculated, but these are not shown. With hypothalamus measurements two-way ANOVA with factors group and drug treatment yielded drug main effects for DOPAC $F(1,26) = 10.92, p < 0.01$, 5-HT $F(1,27) = 23.72, p < 0.001$ and 5-HIAA $F(1,26) = 5.45, p < 0.05$ concentrations and for DOPAC/DA ratio's $F(1,26) = 18.41, p < 0.001$. Post-hoc Student-Newman-Keuls tests showed that in both groups A and B 5-HT concentrations in the hypothalamus were decreased by about 20% in early postnatally clomipramine-treated one-year old rats compared with their respective controls (Fig. 1). 5-HIAA levels were decreased by 20% only in experiment B (Fig. 1). DOPAC concentrations and DOPAC/DA ratio's were increased by about 20% in both groups. Significant interactions between factors group and drug were only present for HVA

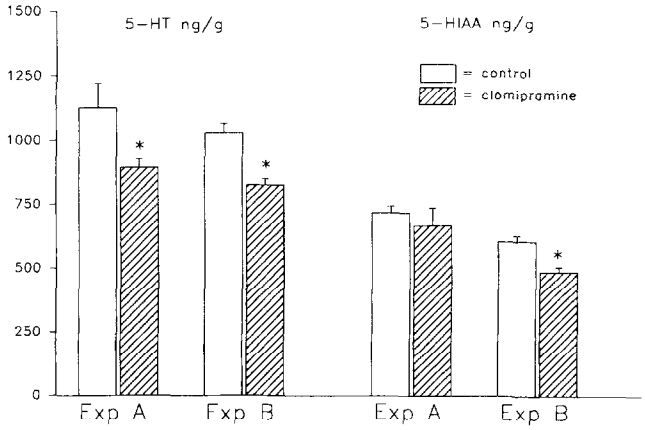

FIG. 1. Concentrations of 5-HT and 5-HIAA in the hypothalamus of one-year old rats that were neonatally treated with clomipramine. Mean values (±SEM) are given of 6–7 (Group A) or 9–10 (Group B) determinations. *Significantly different from controls.

TABLE 2

α_2-ADRENOCEPTOR BINDING IN THE AMYGDALA/PIRIFORM CORTEX

Individual Values		
Control (n = 9)	122 ± 16 fmol/mg protein	
Clomipramine (10)	134 ± 8	
Saturation Analysis	**B_{max}**	**K_D**
Control (n = 4)	151 ± 8 fmol/mg protein	3.2 ± 0.3 nmol/l
Clomipramine (5)	147 ± 7	3.0 ± 0.2

All values are means ± SEM.

Individual values: binding of 10 nmol/l [^{3}H]RX821002 to membrane preparations of individual animals. Saturation analysis: binding of 0.3–10 nmol/l [^{3}H]RX821002 to membrane preparations pooled from 2–3 rats.

concentrations $F(1,27) = 5.02, p < 0.05$ and HVA/DA ratio's $F(1,27) = 5.31, p < 0.05$, which were, however, not found to be different from their respective controls.

With medulla-pons measurements similar analyses showed a drug main effect for DOPAC concentrations $F(1,24) = 4.60, p < 0.05$ and a marginal effect for DOPAC/DA ratio's $F(1,24) = 3.52, p = 0.073$. A significant drug × group interaction was found for DOPAC $F(1,24) = 5.05, p < 0.05$. Both DOPAC concentration and DOPAC/DA ratio were increased in group A only: the ratio by 20% and DOPAC by 30%.

In frontal cortex and amygdala/piriform cortex samples obtained from group A no alterations in the concentrations of monoamines and metabolites were found.

Adrenergic Receptors

No differences were found in the numbers or affinity of α_2-adrenoceptors in the amygdala/piriform cortex region of early postnatally clomipramine-treated rats compared to controls (Table 2). Neither were changes detected in the number or affinity of total β-adrenoceptors or of β-adrenoceptors subtypes in the frontal cortex (Table 3).

DISCUSSION

The important finding of this study is that early postnatal treatment with the antidepressant drug clomipramine leads to adult alterations in monoamine systems in the hypothalamus measured in one year old rats. 5-HT concentrations were decreased and DOPAC levels were increased. As the only other study in which adult hypothalamic levels of monoamines were determined after comparable treatments also reported decreases in hypothalamic 5-HT (14), this might be an important indication for future studies. The fact that some authors consider the functional disturbances in these rats as an animal model for depression (18,43) provides an extra dimension to these studies.

Two groups of animals have been used. Those in group A went through various tests during the time in between the neonatal treatment and the biochemical measurements. Although both groups went through the same food deprivation and weighting schedules, the ones in group B were not used in any test at all and measurements in this group cannot be confounded by the possible neurochemical consequences of the various behavioural tests or physiological measurements, which involved a.o. surgery under general anesthesia. Whether

TABLE 3

β-ADRENOCEPTOR BINDING IN THE FRONTAL CORTEX

	β_{1+2}	β_1	β_2
Individual values			
Control (n = 8)	17 ± 1	13 ± 1	4.7 ± 0.2 fmol/mg prot
Clomipramine (10)	17 ± 1	13 ± 1	4.6 ± 0.2
Saturation analysis			
K_D			
Control (n = 4)	41 ± 6	64 ± 8	14 ± 3 pmol/l
Clomipramine (5)	40 ± 5	69 ± 7	13 ± 3
B_{max}			
Control (n = 4)	20 ± 1	16 ± 1	4.4 ± 0.3 fmol/mg prot
Clomipramine (5)	20 ± 1	17 ± 1	4.5 ± 0.4

All values are means ± SEM.

Individual values: binding of 217 pmol/l [^{125}I]iodocyanopindolol to membrane preparations from individual animals. Saturation analysis: binding of 6.25–200 pmol/l [^{125}I]iodocyanopindolol to membrane preparations pooled from 2–3 rats.

the decrease in hypothalamic 5-HIAA levels in group B and the increase in DOPAC in the medulla-pons in group A might have something to do with the participation in the tests is at the moment not clear. The changes in hypothalamic 5-HT and DOPAC concentrations were present in the clomipramine-exposed rats of both groups A and B when compared to their respective controls and we conclude that these changes are related to the neonatal treatment.

Our present findings are corroborated by the results of the few studies that have been carried out in this direction until now. Hilakivi et al (14) treated rats neonatally with two other antidepressants, desipramine and zimeldine. These rats (of the Long Evans strain) were in adulthood tested for open field behaviour and voluntary alcohol drinking. Subsequent neurochemical measurements (approximately at 6 months of age) in several regions showed the most conspicuous alterations to be present in the hypothalamus, where 5-HT was decreased to 50% and 5-HIAA to 80%. These decreased 5-HT concentrations in combination with decreased or unchanged metabolite levels might tentatively be explained by a decreased serotonergic innervation of the hypothalamus. It is noteworthy that Edwards et al (5) observed a selective decrease in 5-HT uptake sites labeled with [^{3}H]paroxetine in the hypothalamus of adult rats of the Sprague Dawley strain in another proposed animal model for depression, i.e. learned helplessness. Thus the decrease in markers for 5-HT innervation of the hypothalamus has now been observed in two different experimental conditions that have been proposed as animal models of depression, using 3 different rat strains. In the very first paper on the adult effects of neonatal clomipramine exposure, Mirmiran et al (20) hypothesized that a relatively low level of serotonergic inhibition would be involved in some of the reported functional alterations and mentioned the increased incidence of REM sleep and the abnormal male sexual behaviour in this respect (20.27,28). Alterations in hypothalamic monoamines might also be suggested to be related to other functional changes that have been reported after neonatal antidepressant exposure: the alterations in circadian rhythmicity, which were reported after neonatal treatment with another antidepressant drug, desipramine (24); the decreased corticosteroid activation by stress in adult rats that were neonatally treated with clomipramine (22). Alterations in serotonergic activity have also been reported: the unit activity of 5-HT neurons of neonatally clomipramine-treated rats was decreased (30). However, Maudhuit et al (18) found a normal firing rate but a reduction in the inhibitory response to a 5-HT reuptake blocker, indicating a defective negative feedback mechanism.

In both our and Hilakivi's studies either DA or DOPAC concentrations were changed in a way that resulted in increased hypothalamic DA metabolite/transmitter ratios. Whether this would indicate a locally increased DA activity in these animals can only be concluded after additional studies using other techniques. A further similarity is that in both studies no changes were found in monoamine metabolism in the frontal cortex and no consistent changes in the medulla-pons region. Also in another study (10) no changes were detected in uptake and spontaneous and evoked release of 5-HT in frontal cortex of adult rats postnatally exposed to zimeldine. It is not clear why hypothalamic monoaminergic, in particular serotonergic, systems apparently are more affected than monoamines in other areas. It might be that clomipramine effects in the hypothalamus result in local interactions between hypothalamic neurons and monoaminergic terminals or neurons, leading to long-lasting alterations.

Taken together, it is clear that chronic neonatal treatment with antidepressants results in lasting changes in the serotonergic and dopaminergic systems in the hypothalamus. At present, we do not know whether the neurochemical changes are related to the physiological and behavioural disturbances that led some authors to propose this as a model for depression, and if so, whether they underlie the functional alterations or are the consequence of them. A comprehensive study involving neurochemical and functional measurements at various times during development and adulthood after the neonatal treatment might provide an answer to this. In the present study no changes were detected in the numbers of β- and α_2-adrenoceptors that lasted into adulthood. This is in line with our previous studies that changes in adrenergic receptors induced by early postnatal drug treatments do not last for a long time (7), although it has been suggested that alterations of serotonergic and adrenergic receptor numbers are induced more readily and for a longer period of time during development than in adulthood (4).

In conclusion, we have shown that some consistent changes in adult neurochemical parameters are found in the hypothalamus of rats that have been treated neonatally with clomipramine or other antidepressants. Although it is difficult to directly relate the decrease in hypothalamic 5-HT and increase in hypothalamic DOPAC to functional disturbances in adult animals that were neonatally exposed to clomipramine, the nature of the disturbances suggest that hypothalamic 5-HT could well be involved.

REFERENCES

1. Boer, G.J., Van der Hee, R. and Feenstra, M.G.P., Alpha-2-adrenoceptor assayed in rat brain by the new ligand [^{3}H]RX821002, Biog Amines, 9 (1993) 259–269.
2. Coyle, J.T. and Axelrod, J., Development of the uptake and storage of L-[^{3}H]norepinephrine in the rat brain, J. Neurochemistry, 18 (1971) 2061–2075.
3. De Boer, S., Mirmiran, M., Van Haaren, F., Louwerse, A. and Van de Poll, N.E., Neurobehavioral teratogenic effects of clomipramine and alpha-methyldopa. Neurotoxicol. Teratol., 11 (1989) 77–84.
4. Del Río, J., Montero, D., De Ceballos, M.L., Long-lasting changes after perinatal exposure to antidepressants. In: G.J. Boer, M.G.P. Feenstra, M. Mirmiran, D.F. Swaab and F. Van Haaren (Eds.), Biochemical basis of functional neuroteratology. Permanent effects of chemicals on the developing brain, Progress in Brain Research, Vol. 73, Elsevier, Amsterdam, 1988, pp 173–187.
5. Edwards, E., Harkins, K., Wright, G. and Henn, F., Modulation of [^{3}H]paroxetine binding to the 5-hydroxytryptamine uptake site in an animal model of depression, J. Neurochem., 56 (1991) 1581–1586.
6. Erdtsieck-Ernste, E.B.H.W., Feenstra, M.G.P. and Boer, G.J., Pre- and postnatal developmental changes of adrenoceptor subtypes in rat brain, J. Neurochem., 57 (1991) 897–903.
7. Erdtsieck Ernste, E.B.H.W., Feenstra, M.G.P. and Boer, G.J., Perinatal influence of β-adrenergic drugs on the noradrenergic system of the rat brain, Gen. Pharmacol., 24 (1993) 1069–1078.
8. Feenstra, M.G.P., Snijdewint, F.G.M., Van Galen, H. and Boer, G.J., Widespread alterations in central noradrenaline, dopamine,

and serotonin systems in the Brattleboro rat not related to the local absence of vasopressin, Neurochem. Res., 15 (1990) 283–288.
9. File, S.E. and Tucker, J.C., Neonatal clomipramine treatment in the rat does not affect social, sexual and exploratory behaviors in adulthood, Neurobehav. Toxicol. Teratol. 5 (1983) 3–8.
10. Grimm, V.E. and Frieder, B., Prenatal and early postnatal exposure to zimelidine: behavioral, neurochemical and histological findings in rats, Int. J. Neurosci., 33 (1987) 225–235.
11. Heninger, G.R. and Charney, D.S., Mechanism of action of antidepressant treatments: implications for the etiology and treatment of depressive disorders, In H.Y. Meltzer (Ed.) Psychopharmacology: The third generation of progress, Raven Press, New York, 1987 pp 535–544.
12. Hilakivi, L.A. and Hilakivi, I., Increased adult behavioral despair in rats neonatally exposed to desipramine or zimeldine: an animal model of depression?, Pharmacol. Biochem. Behav., 28 (1987) 367–369.
13. Hilakivi, L.A., Hilakivi, I., Ahtee, L., Haikala, H. and Attila, M., Effect of neonatal nomifensine exposure on adult behavior and brain monoamines in rats, J. Neural Transm., 70 (1987) 99–116.
14. Hilakivi, L.A., Stenberg, D., Sinclair, J.D. and Kilanmaa, K., Neonatal desipramine or zimeldine treatment causes long-lasting changes in brain monoaminergic systems and alcohol related behavior in rats, Psychopharmacol., 91 (1987) 403–409.
15. Hilakivi, L.A., Taira, T., Hilakivi, I. and Loikas, P., Neonatal treatment with monoamineuptake inhibitors alters later response in behavioural 'despair' test to β and GABA-B receptor agonists, Pharmacol. Toxicol., 63 (1988) 57–61.
16. Hyttel, J., Citalopram - pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity, Prog. Neuro-Psychopharmacol. & Biol. Psychiat. 6 (1982) 277–295.
17. Karki, N., Kuntzman, R. and Brodie, B.B., Storage, synthesis and metabolism of monoamines in the developing brain, J. Neurochemistry, 9 (1962) 53–58.
18. Maudhuit, C., Hamon, M. and Adrien, J., Electrophysiological activity of raphe dorsalis serotoninergic neurones in a possible model of endogenous depression, NeuroReport 6 (1995) 681–684.
19. McPherson, G.A., Analysis of radioligand binding experiments. A collection of computer programs for the IBM PC, J. Pharmacol. Methods, 14 (1985) 213–228.
20. Mirmiran, M., Van de Poll, N.E., Corner, M.A., Van Oyen, H.G. and Bour, H.L., Suppression of active sleep by chronic treatment with chlorimipramine during early postnatal development: effects upon adult sleep and behavior in the rat, Brain Res., 204 (1981) 129–146.
21. Mirmiran, M., Scholtens, J., Van de Poll, N.E., Uylings, H.B.M., Van der Gugten, J. and Boer, G.J., Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat, Devel. Brain Res., 7 (1983) 277–286.
22. Ogawa, T., Mikuni, M., Kuroda, Y., Muneoka, K., Mori, K.J. and Takahashi, K., Effects of the altered serotonergic signalling by neonatal treatment with 5,7-dihydroxytryptamine, ritanserin or clomipramine on the adrenocortical stress response and the glucocorticoid receptor binding in the hippocampus in adult rats, J. Neural Transm. 96 (1994) 113–123.
23. Racagni, G., Mocchetti, I., Brunello, N., Renna, G. and Cuomo, V., Early biochemical and behavioral changes after prolonged exposure to antidepressant drugs. In G. Zbinden, V. Cuomo, G. Racagni, B. Weiss (Eds.) Application of behavioral pharmacology in toxicology, Raven Press, New York, 1983, pp 161–171.
24. Rosenwasser, A.M. and Hayes, M.J., Neonatal desipramine treatment alters free-running circadian drinking rhythms in rats, Psychopharmacol. 115 (1994) 237–244.
25. Ugrumov, M.V., Proshlyakova, E.V. and Sapronova, A., Development of the hypothalamic 5-hydroxytryptamine system during ontogenesis in rats: uptake and release of 5-hydroxytryptamine in vitro, Neuroscience, 32 (1989) 127–131.
26. Velazquez-Moctezuma, J. and Diaz Ruiz, O., Neonatal treatment with clomipramine increased immobility in the forced swim test: an attribute of animal models of depression, Pharmacol. Biochem. Behav., 42 (1992) 737–739.
27. Velazquez-Moctezuma, J., Aguilar-Garcia, A. and Diaz-Ruiz, O., Behavioral effects of neonatal treatment with clomipramine, scopolamine, and idazoxan in male rats, Pharmacol. Biochem. Behav., 46 (1993) 215–217.
28. Vogel, G., Neill, D., Hagler, M. and Kors, D., A new animal model of endogenous depression: a summary of present findings. Neurosci. Biobehav. Rev., 14 (1990) 85–91.
29. Westerink, B.H.C., Analysis of trace amounts of catecholamines and related compounds in brain tissue: a study near the detection limit of liquid chromatography with electrochemical detection, J. Liq. Chromatogr., 6 (1983) 2337–2351.
30. Yavari, P., Vogel, G.W. and Neill, D.B., Decreased raphe unit activity in a rat model of endogenous depression, Brain Res., 611 (1993) 31–36.