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Prenatal Stress: Effect on Development of Rat Brain Adrenergic Receptors

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PETERS, D. A. V. *Prenatal stress: Effect on development of rat brain adrenergic receptors.* PHARMACOL BIOCHEM BEHAV 21(3) 417–422, 1984.—Female rats were subjected to stress treatments during pregnancy and the offspring were studied at several different ages. The ligands [^{3}H] WB-4101, [^{3}H]clonidine and [^{3}H]dihydroalprenolol were used to measure α1, α2 and β receptor binding in several brain regions. At 16 but not at 23, 40 or 60 days of age the offspring showed reduced α1 and β receptor binding in cerebral cortex whereas a previous study had shown a similarly transient elevation of norepinephrine (NE) level at 16 days of age. The 60 day-old offspring showed only a reduced α2 binding which appeared to have no regional specificity. Consistent with our previous finding that in 60 day-old offspring NE levels were not significantly affected by maternal stress exposure in almost all brain regions studied, we find no effect on the ability of nerve endings to synthesise catecholamines. These data provide additional support for the proposal that prenatal stress results in permanent neurochemical changes and suggest that there may be a delayed or impaired development of the postsynaptic elements of noradrenergic neurons.

Stress, prenatal Adrenergic receptors Tyrosine hydroxylation

MANY studies have provided evidence that the offspring of rats exposed to stress treatments during pregnancy show behavioral deficits at maturity. Some evidence suggests that persistent biochemical changes involving catecholamine-containing neurons may be associated with behavioral deficits. For example, Moyer *et al.* [7] found changes in norepinephrine (NE) and dopamine levels in several discrete brain regions from adult offspring of stressed rats and suggested that prenatal stress may result in permanent neurochemical changes responsible for feminisation of male offspring. Similarly, we previously reported [8] that prenatal stress increased the NE level in the hypothalamus of 60 day-old rats but had no apparent effect in 5 other brain regions.

Although prenatal stress exposure has only a limited effect on brain NE levels in the adult, our previous study provided evidence that there may be more widespread changes in the early postnatal development of central noradrenergic neurons. Thus, in both cerebral cortex and pons-medulla prenatal stress treatment resulted in significant changes in NE level during the neonatal period of rapid development of monoaminergic neurons but not older animals [8].

The absence of changes in NE level in most brain regions of adult offspring does not necessarily indicate that there had been no effect on the functioning of noradrenergic neurons. We therefore re-examined the effect of prenatal stress on central noradrenergic neurons using other biochemical measures to detect possible changes. The binding of [^{3}H]-labelled adrenergic ligands to brain membrane was used to study adrenergic receptor binding while the hydroxylation of [^{14}C]-labelled tyrosine in brain homogenates containing intact synaptosomes was used as a measure of the ability of nerve endings to synthesise catecholamines [4]. We now report that prenatal stress reduced both α1 and β receptor binding in cerebral cortex at 16 days of age but not at 23, 40 or 60 days of age whereas α2 binding was significantly reduced in the 60 day-old offspring but not in younger animals. Tyrosine hydroxylation in nerve endings was unimpaired in 60 day-old offspring of stressed rats. These results provide additional evidence that prenatal stress influences the development of noradrenergic neurons and it is suggested that this effect may be involved in the mechanism responsible for behavioral deficits found in prenatally stressed rats.

METHOD

Animals

Adult Sprague-Dawley rats were maintained in the animal care facilities of the University of Ottawa Health Sciences Center in a temperature and humidity controlled room on a 12 hr light, 12 hr dark cycle for an acclimatisation period of 2 weeks before mating. One male rat was then placed in each cage containing 3 female rats for a 4 day period. After removal of the males the cages were randomly assigned to either the control or the stress group. A total of 16 female rats in the stress group received daily stress treatments from the day of separation until birth of the litters appeared to be imminent. Stress treatments consisted of once daily removal of the cages to a nearby laboratory where each rat was given a single saline injection (0.02 ml, SC) in randomised order [7]. An equal number of control rats remained throughout pregnancy in the animal room. No attempt was made to verify fertilisation in either group to avoid the stress involved [12].

All female rats were transferred to individual breeding cages shortly before giving birth. Within 12 hours of birth the litters were quickly weighed and then reduced to 10 pups. At 16 days of age one male and one female pup were removed from each litter, immediately transferred to the nearby laboratory, killed by decapitation and the brains removed. The cerebellum, cerebral cortex, corpus striatum, pons-medulla, hippocampus and spinal cord were dissected out on a cooled glass plate, weighed, frozen in liquid nitrogen and stored in individual polypropylene capsules at −80°.

The remaining pups were weaned at 22 days and separated by treatment group and sex. A further male-female pair were taken from each litter at 23 and 40 days of age and brain parts were prepared and stored as before. All remaining animals were similarly treated at 60 days of age except for 1 set of animals where brain tissues were used immediately for the tyrosine hydroxylation assays. All animals were killed in the same 3 hr period (9 a.m.–11 a.m.) to minimise the effect of circadian rhythms on receptor binding [3].

When tissues were assigned to the various assays care was taken to ensure that the largest number of different litters were represented in each experiment. Tissues from more than 1 animal per litter were used in a single experiment only when it was necessary to combine tissues.

Reagents

[^{3}H]WB-4101 (25.7 ci/mMol), [^{3}H]clonidine (24 Ci/mMol), [^{3}H]dihydroalprenolol ([^{3}H]DHA; 52 Ci/mMol), L-[U-^{14}C]tyrosine (460 mCi/mMol) and L-[l-^{14}C]tyrosine (54 mCi/mMol) were obtained from Amersham Corporation or New England Nuclear.

Binding Assays

Brain tissues were homogenised in 20 volumes of ice-cold 50 mM tris-HCl buffer, pH 7.7, using a Brinkman Polytron PT-10. The homogenates were centrifuged at 50000 × g for 20 min, the pellet resuspended in the same volume of fresh buffer, recentrifuged, and the final pellet dispersed in 60 volumes of buffer.

The techniques used to study α1, α2 and β receptor binding were closely similar to those described by U'Prichard *et al.* [13–15]. The specific binding of [^{3}H]WB-4101 to brain tissue was used as a measure of α1 adrenergic receptor binding [13]. For the assay at a single concentration of [^{3}H]-labelled ligand, incubation tubes contained 0.8 ml of the tissue suspension and 0.2 ml of a solution of [^{3}H]WB-4101 to give a final concentration of 0.5 nM. The incubations were carried out in triplicate in parallel with a second set of tubes which also contained a 20 μM concentration of (−)-NE to define non-specific binding. The tubes were incubated for 30 min at 25° after which the contents were rapidly filtered under vacuum through Whatman GF/B filters. After rinsing tubes and filters 3 times with 5 ml aliquots of cold 50 mM tris-HCl buffer, pH 7.7, the filters were placed in vials containing 10 ml PCS (Amersham Corporation) and the associated radioactivity was measured in a Beckman LS 8100 liquid scintillation system at better than 40% efficiency. Specific binding was calculated as the difference in binding in the presence and absence of (−)-NE.

The methods used to study [^{3}H]clonidine [15] and [^{3}H]DHA [14] binding were essentially the same as that described for [^{3}H]WB-4101. For the measurement of α2 receptor binding the incubation tubes contained [^{3}H]clonidine at a final concentration of 1 nM with 1 μM unlabelled clonidine to define non-specific binding. Similarly, for the study of β receptor binding the tubes contained 0.5 nM [^{3}H]DHA with 10 μM isoproterenol to define non-specific binding.

To provide data for Scatchard plots, 8–10 different concentrations of [^{3}H]-labelled ligand in the range of 0.05–2 nM for [^{3}H]WB-4101, 0.2–4.0 nM for [^{3}H]clonidine and 0.2–2.0 nM for [^{3}H]DHA were used. The cerebral cortices from 2 rats were combined to provide sufficient tissue to carry out determinations of all 3 populations of receptors in the same tissue sample. Regression analyses were used to determine values of K_D and Bmax for each Scatchard plot and the results were calculated as the Mean±S.E.M. for 5 separate determinations.

Tyrosine Hydroxylation

Brain tissues were homogenised in 20 vol ice-cold 0.32 M sucrose immediately after dissection. Each homogenate was centrifuged at 40,000 g for 30 min, the pellet washed once with the same volume of fresh 0.32 M sucrose and the final pellet resuspended in 15 vol 0.32 M sucrose. This preparation was used to measure tyrosine hydroxylation by 2 different procedures, firstly the method of McGeer *et al.* [5] in which L-[^{14}C] dopa formed from L-[^{14}C] tyrosine is isolated on an alumina column and secondly the method of Weiner [18] as modified by Kuczenski and Segal [4] in which the release of $^{14}CO_2$ from L-[1-^{14}C] tyrosine is measured.

Duplicate 0.1 ml aliquots of the tissue suspension were incubated at 37° for 30 min with 0.2 ml of a solution containing 0.14 M sodium phosphate, 1.5 mM NSD 1034 and 3 μM L-[U^{14}C] tyrosine (diluted with cold carrier to specific activity 50 mCi/mmol) at a pH of 6.2. Blanks consisted of the same incubation mixture except that the tissue suspension was heated in a boiling water bath for 5 min and cooled before addition of the remainder of the mixture. The reaction was stopped with 2 ml of a 1:1 mixture of 0.4 M perchloric acid and 0.2 acetic and the L-[^{14}C] dopa formed was isolated on an alumina column according to the procedure described by McGeer *et al.* [5]. Radioactivity was assayed in a Beckman LS 8100 liquid scintillation system.

In one experiment synaptosomal tyrosine hydroxylation was assayed according to the procedure described by Kuczenski and Segal [4] in which $^{14}CO_2$ released during the decarboxylation stage of catecholamine synthesis is trapped in NCS and counted by liquid scintillation spectrometry. This assay is based on the finding that in intact synaptosomes any L-[^{14}C] dopa formed by tyrosine hydroxylation is quickly decarboxylated to dopamine.

RESULTS

Litters

Twelve control and 10 prenatal stress litters with 10 or more pups each were obtained. An additional control litter of 6 pups and a stress litter with 4 pups were discarded. Prenatal stress litters appeared to be slightly larger than control litters (control: males 5.3±0.8, females 5.2±0.8; Stress: males 6.6±0.5, females 5.9±0.5) while birth weights appeared to be correspondingly reduced (control: males 7.28±0.22 g, females 7.40±0.13 g; Stress: males 6.48±0.43 g, females 6.34±0.29 g) but the values were not significantly different when tested by ANOVA.

Biochemistry

Scatchard plots of binding data for [^{3}H]WB-4101,

TABLE 1

EFFECT OF PRENATAL STRESS ON [^{3}H]WB-4101, [^{3}H]CLONIDINE AND [^{3}H]DHA BINDING IN CEREBRAL CORTEX AND PONS-MEDULLA OF 60 DAY-OLD MALE RATS

	Treatment	Kd (nm)	Bmax (fmol/mg protein)	Bmax (%)
[^{3}H]WB-4101				
Cortex	Control	0.30 ± 0.03	93 ± 4	
	Stress	0.29 ± 0.05	93 ± 6	0
Pons-medulla	Control	0.24 ± 0.04	66 ± 4	
	Stress	0.28 ± 0.05	69 ± 3	+5
[^{3}H]clonidine				
Cortex	Control	1.9 ± 0.5	135 ± 5	
	Stress	1.9 ± 0.4	101 ± 6*	−25
Pons-medulla	Control	1.9 ± 0.4	46 ± 4	
	Stress	2.2 ± 0.5	35 ± 3*	−24
[^{3}H]DHA				
Cortex	Control	0.40 ± 0.03	78 ± 6	
	Stress	0.43 ± 0.06	83 ± 7	+6
Pons-medulla	Control	0.44 ± 0.05	52 ± 4	
	Stress	0.47 ± 0.03	53 ± 3	+2

Scatchard plots were prepared from binding data obtained using 8–10 different ligand concentrations (0.05–2 nM for [^{3}H]WB-4101, 0.2–4.0 nM for [^{3}H]clonidine and 0.2–2.0 nM for [^{3}H]DNA). Tissues from 2 rats were pooled to provide tissue for all 3 assays. The values shown are mean ± S.E.M. for 5 determinations of K_D and Bmax using tissues from 8 different litters in each group.

*Denotes $p<0.05$ by t-test.

[^{3}H]clonidine and [^{3}H]DHA in cerebral cortex and pons-medulla of 60 day-old rats were linear over the range of ligand concentrations employed. Maternal stress did not alter the apparent dissociation constants (K_D's) and the only significant effect on receptor density (Bmax) proved to be a decrease in [^{3}H]clonidine binding in cerebral cortex (Table 1).

The binding of the same [^{3}H] labelled ligands was also studied in 5 brain regions of 60 day-old offspring using a single concentration of each ligand (Table 2). When tested by a 2-way ANOVA the data showed a highly significant treatment effect for [^{3}H]clonidine binding ($p<0.001$) with no treatment × region interaction ($p>0.1$). As before, the binding of [^{3}H]WB-4101 and [^{3}H]DHA appeared to be unaffected by prenatal stress exposure.

We also examined the ligand binding in the cerebral cortices of 16, 23 and 40 day-old offspring of control and stressed rats (Table 3). Two-way ANOVA's on the 16, 23 and 40 day data combined with the 60 day data for cerebral cortex presented in Table 2 showed significant treatment × age effects for all three ligands. Further analysis by t-test showed significant reductions in [^{3}H]WB-4101 and [^{3}H]DHA binding at 16 days of age with no effect at either 23, 40 or 60 days of age.

We found no effect of prenatal stress on the ability of sucrose homogenates of brain parts to synthesise L-[^{14}C]dopa from L-[^{14}C]tyrosine using 6 brain regions from 60 day-old male offspring (Table 4). Similarly when the release of $^{14}CO_2$ from L-[1-^{14}C]tyrosine was used as a measure of tyrosine hydroxylation prenatal stress treatment was without apparent effect (data not shown).

DISCUSSION

There is evidence that [^{3}H]WB-4101 binds to α1 adrenergic receptor sites while [^{3}H]clonidine mainly labels the α2 sites in the central nervous system [15–16]. The α2 sites may be associated with presynaptic autoreceptors on noradrenergic terminals in the peripheral nervous system but some evidence suggests a postsynaptic location in the brain. For example, 6-hydroxydopamine treatment increases both high and low affinity binding in almost all brain regions [15], consistent with the hypothesis of postsynaptic receptor "super-sensitisation" following lesioning of the NE terminals. However, it may still be possible that a portion of the sites labelled by [^{3}H]clonidine represent presynaptic autoreceptors. Although [^{3}H]DHA appears to label both β1 and β2 adrenergic sites in most brain regions the β receptors are principally, usually >80%, of the β1 subtype [6]. It is also probable that the smaller β2 population is associated with non-neuronal structures in the brain [6]. Both the α1 and β1 receptors appear to be primarily located post-synaptically [11,13].

We now report that at 16 days of age there were significant reductions in the binding of [^{3}H]WB-4101 and [^{3}H]DHA to a cerebrocortical membrane preparation suggesting that the densities of α1 and β receptors had been reduced by prenatal stress.

Several studies have found different time courses for the development of pre- and postsynaptic components of the noradrenergic system and the development of postsynaptic β receptors appear to be independent of the presynaptic

TABLE 2

MATERNAL STRESS: EFFECT ON ADRENERGIC RECEPTOR BINDING IN BRAIN REGIONS OF 60 DAY-OLD MALE OFFSPRING

		Specific binding (fmol/g protein)				
		Cerebellum	Cortex	Hippocampus	Pons-medulla	Straitum
[^{3}H]WB-4101	Control	23.2 ± 1.8	74.8 ± 4.0	47.0 ± 5.8	50.0 ± 2.6	40.2 ± 2.2
(0.5 nM)	Stress	23.0 ± 2.0	83.2 ± 3.6	50.2 ± 3.8	47.2 ± 6.2	40.8 ± 3.2
		(99)†	(111)	(107)	(114)	(101)
[^{3}H]Clonidine	Control	7.3 ± 0.7	43.0 ± 2.0	18.8 ± 1.6	14.6 ± 1.6	16.5 ± 1.2
(1.0 nM)	Stress	5.4 ± 0.4*	37.0 ± 1.2	18.4 ± 2.0	10.8 ± 1.2	11.4 ± 1.8*
		(74)	(86)	(98)	(74)	(69)
[^{3}H]DHA	Control	16.6 ± 1.4	36.4 ± 2.5	35.4 ± 2.1	25.4 ± 2.6	37.4 ± 6.0
(0.5 nM)	Stress	19.0 ± 1.5	39.0 ± 3.7	32.2 ± 2.6	25.7 ± 1.5	39.8 ± 5.1
		(115)	(102)	(91)	(101)	(106)

Data is for 8 control and 8 prenatal stress rats, each from a different litter. Specific binding of [^{3}H]WB-4101, [^{3}H]clonidine and [^{3}H]DHA was defined as the difference in binding in the presence and absence of 20 μM (−)NE, 1 μM clonidine and 10 μM isoproterenol respectively. Assays were carried out in triplicate and all 3 assays were performed on the same homogenate.

*Denotes $p<0.05$ by t-test.

†Percentage of control value.

TABLE 3

MATERNAL STRESS: EFFECT ON ADRENERGIC RECEPTOR BINDING IN CEREBRAL CORTEX OF MALE OFFSPRING

Age (days)	Treatment	[^{3}H]WB-4101 fmol/mg protein	[^{3}H]Clonidine fmol/mg protein	[^{3}H]DHA fmol/mg protein
16	Control	25.6 ± 2.2	17.6 ± 3.4	29.4 ± 4.4
	Stress	18.8 ± 2.2*	14.0 ± 2.3	14.0 ± 2.8*
	% control	73	80	48
23	Control	65.2 ± 8.0	31.3 ± 4.7	33.7 ± 4.1
	Stress	74.1 ± 9.1	34.8 ± 4.6	33.4 ± 1.6
	% control	114	111	99
40	Control	77.4 ± 4.0	31.4 ± 3.4	36.6 ± 4.4
	Stress	88.7 ± 5.5	31.4 ± 5.7	40.2 ± 3.3
	% control	115	100	110

Each value was obtained from a group of 8 rats, each taken from a different litter. Assays were carried out in triplicate and all 3 assays were performed on the same homogenate. Specific binding of [^{3}H]WB-4101 (0.5 nM), [^{3}H]clonidine (1.0 nM) and [^{3}H]DHA (0.5 nM) was defined as the difference in binding in the presence and absence of 20 μM (−)-NE, 1 μM clonidine and 10 μM isoproterenol respectively.

*Denotes $p<0.05$ by t-test.

TABLE 4

PRENATAL STRESS: EFFECT ON TYROSINE HYDROXYLATION IN BRAIN HOMOGENATES

	L-[^{14}C] Dopa formed (nmoles/g wet weight/hr)	
	Control	Stress
Cerebellum	0.48 ± 0.03	0.50 ± 0.03
Cortex	1.45 ± 0.11	1.53 ± 0.09
Hippocampus	0.95 ± 0.08	0.95 ± 0.06
Pons-medulla	1.14 ± 0.04	1.12 ± 0.07
Spinal cord	0.80 ± 0.04	0.81 ± 0.04
Striatum	40.7 ± 1.1	41.2 ± 1.7

Results are mean ± S.E.M. for groups of 8 male rats each from a different litter. The rate of tyrosine hydroxylation was measured as the conversion of L-[^{14}C] tyrosine to L-[^{14}C] dopa in the presence of a decarboxylase inhibitor.

neurons [2,9] although the same may not be true for α receptors [1]. If the maturation of postsynaptic elements of NE synapses is not strongly influenced by growth of the presynaptic structures then a possible explanation of our data is that the development of NE target cells is retarded in prenatally stressed animals. It is therefore of interest that the decrease in β receptor binding (-52%, $p<0.01$) appeared to be more pronounced than the decreases in either $\alpha 1$ (-27%, $p<0.05$) or $\alpha 2$ (-20%, $p>0.05$) binding at 16 days, consistent with the view that development of β receptor binding at least is independent of the presynaptic system.

The reduction in receptor binding at 16 days only correlates with our previous finding of increased cortical NE levels at 9 and 16 days but no change at 23 days of age [8]. A possible explanation of these findings is that there is a transitory period between about 9 and 23 days of age during which synthesis and release of NE is stimulated as part of a compensatory mechanism triggered by the reduced density of postsynaptic noradrenergic receptors. The return of NE levels and receptor binding to control values between 16 and 23 days may reflect the delayed maturation of the NE target cells and possibly the development of compensatory changes in other neurotransmitter systems. Our previous finding that 5-HT receptor binding in the cerebral cortex of prenatally stressed rats increased relative to control values at about this age suggests that serotonergic neurons may be involved in the compensatory changes (submitted for publication). A slightly different explanation is suggested by the results of recent investigations which have supported the hypothesis that NE may influence neuronal development through a cycle-AMP-mediated mechanism in postsynaptic neurons [17]. It is conceivable that the increased NE levels at 16 days of age may be involved in the mechanism by which the delayed increase in postsynaptic receptor numbers occurs.

In mature offspring, the only significant effect of prenatal stress treatment in this study was a reduced [^{3}H]clonidine binding in all brain regions studied with the possible exception of the hippocampus. Since the location and functions of the [^{3}H]clonidine binding sites have not been unequivocally established for the central nervous system the significance of the decrease is unknown. However, if [^{3}H]clonidine does in part label the presumed presynaptic autoreceptors then the decrease in binding might represent a reduction in the number of these receptors. Such a situation could reflect a modified regulation of neurotransmitter release, resulting in an altered NE turnover. We therefore decided to investigate whether prenatal stress had any effect on the rate of synthesis of catecholamines in nerve endings.

Tyrosine hydroxylase, the presumed rate-limiting enzyme in the biosynthesis of catecholamines, exists in both soluble and particulate forms and evidence has been presented that nerve endings can be distinguished by the presence of the particulate enzyme [4,10]. The rate of tyrosine hydroxylation measured in the absence of exogenous cofactors in preparations containing intact synaptosomes may provide a more useful measure of the ability of nerve terminals to synthesise catecholamines than would an assay of total tyrosine hydroxylase [4]. Our failure to find changes in tyrosine hydroxylation in various brain regions following the prenatal stress treatment therefore suggests that there was no alteration in the overall rate of synthesis of catecholamines in nerve endings. This finding suggests that the decrease in [^{3}H]clonidine binding is not associated with a change in neurotransmitter synthesis or release.

In summary, this report of significant reductions in α and β receptor binding in cerebral cortex at the age at which we had previously found a significant increase in NE level provides additional evidence that prenatal stress exposure affects the postnatal development of noradrenergic neurons. Moreover, the finding of increased $\alpha 2$ receptor binding in several brain regions in the adult offspring is consistent with the view that prenatal stress may have permanent effects on the functioning of the central noradrenergic system and it is possible that such changes may be involved in the origins of the behavioral deficits found in prenatally stressed rats.

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