regulations of animal care and use of the State of Israel. The experiments reported herein were also conducted according to the principles set forth in the Guide for Care and Use of Laboratory Animals, Institute of Laboratory Animal Medicine, National Research Council, DHEW Publication No. (NIH) 80-23, 1985. We wish to thank Mrs Cresson for her expert assistance in preparing this manuscript.

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Stress-induced increase in noradrenaline release in the rat hypothalamus assessed by intracranial microdialysis

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Summary. The hypothalamic microdialysis of conscious rats was used to investigate the effects of immobilization stress (20 min) on extracellular noradrenaline(NA) levels. The stress significantly increased NA levels relative to basal efflux by 106% and this elevation continued for 40 min after release from stress.
Key words. In vivo microdialysis; stress; noradrenaline release; hypothalamus; high performance liquid chromatography; rat.

The activation of central noradrenergic systems by various stressful stimuli has been demonstrated[1], and the marked increase in noradrenaline (NA) turnover has been reported to occur in the hypothalamus of rodents exposed to stress[2, 3]. We have reported that immobilization stress increases NA turnover in various brain regions[4, 5], which show regional differences in terms of both the degree of responsiveness and its time course.

Recently, both the development of sensitive catecholamine assay methods utilizing high performance liquid chromatography (HPLC) and the introduction of the microdialysis technique have permitted the investigation of endogenous catecholamine release from regional brain areas in vivo[6].

In the present study, in order to investigate the time course of endogenous NA release in the hypothalamus in response to immobilization stress, we attempted the direct measurement of extracellular NA levels in the anterior hypothalamus in conscious rats using a microdialysis method.

Materials and methods

Male Wistar rats, weighing 300–350 g, were used to perform the intracranial microdialysis in the anterior hypothalamus according to the method of Badoer et al.[7]. The animals were anesthetized with pentobarbital (45 mg/kg i.p.) and the probe, the tip of which consisted of a U-shaped dialysis membrane, was implanted into the anterior hypothalamus (coordinates: rostral – 1.8 mm, lateral 0.7 mm, ventral 9.0 mm, according to bregma and the dural surface)[8]. Microdialysis was started 24 h after the surgery. The dialysis probe was perfused with an artificial CSF with the following composition: NaCl 140 mM, KCl 3.35 mM, $MgCl_2$ 1.15 mM, $CaCl_2$ 1.26 mM, Na_2HPO_4 1.20 mM, and NaH_2PO_4 0.3 mM, pH 7.4. After the experiment, the position of the dialysis probe was verified by anatomical examination. The dialysate (34 µl/20 min) was injected directly into the HPLC-ECD system (EICOM Co., Kyoto, Japan) for measurement of NA. The mobile phase in the HPLC was 0.1 M sodium dihydrogen phosphate buffer (pH 3.1) containing 2.27 mM octanesulfonic acid, 0.05 mM EDTA and 9.0% (v/v) methanol. The column, 25 cm 7 µm ODS-C18 resin (EICOM Co., Kyoto, Japan), was used at 30 °C. The flow rate (EICOM EP-10 pump) was 0.6 ml/min and the graphite working electrode was set at + 600 mV versus a Ag/AgCl reference electrode (EICOM ECD-100 electrochemical detector).

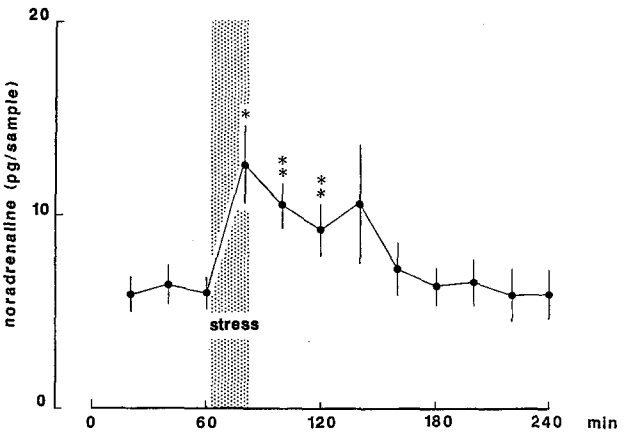
After a stable basal NA level had been obtained for the dialysate (1 to 2 h after the beginning of the perfusion), the animal was stressed by immobilization with a flexible wire mesh for 20 min, as previously reported[4]. Sample collection was continued for at least 4 h. Each fraction was collected for 20 min. Statistical significance was evaluated using the paired Student's t-test. The averaged basal levels of NA were determined from the mean of 3 samples prior to stress. The effect of restraint was evaluated as the difference between the average of the basal values and the levels measured during and after the stress. All values are expressed as the mean ± SEM of 5–6 rats.

Results

The basal level of NA measured in 20-min fractions in the hypothalamic dialysate corresponded to 5.47 ± 0.37 pg/20 min (n = 16). As shown in the figure, immobilization stress for 20 min caused a significant increase in NA release (11.47 ± 1.85 pg/20 min, n = 6). The NA level in the dialysate obtained from 40 min after the release from stress was still significantly higher than the basal level. Two hours after the beginning of stress, NA levels recovered to basal levels.

Discussion

The present study demonstrated that microdialysis is a useful method by which to investigate changes in endogenous NA release in the hypothalamus. Badoer[7] has shown that NA release in the hypothalamus, detected by this technique, increased according to increasing K^+ content in the perfusion fluid, and that the K^+-induced increase in amine release was attenuated under conditions of Ca^{++} depletion. Furthermore, NA output, studied by this method, was reported to be Ca^{++}-dependent and to be increased and decreased by idazoxan and clonidine, respectively[9]. These findings indicate that the measured NA from the dialysate might originate from transmitter released from nerve terminals.



Effect of immobilization stress on hypothalamic noradrenaline release in vivo. Before the fourth sample was taken, the animal was exposed to immobilization for 20 min. Each sample represents a 20-min collection period. The value indicates the mean ± SEM of 5–6 rats. Statistically significant as compared to basal values; $*p < 0.05$; $**p < 0.01$.

The extracellular NA level increased significantly after immobilization stress. This result indicates that NA release is increased in this region by immobilization stress. From the facts that: 1) immobilization caused an increase in the level of 3-methoxy-4-hydroxyphenylethyleneglycol sulfate (MHPG-SO_4) which is the major metabolite of the rat brain NA[10]; 2) MHPG-SO_4 is considered to reflect neuronally released NA[11] in brain regions including the hypothalamus, and 3) that such increases are accompanied by reductions in NA levels in most regions, we suggested that immobilization causes an increase in NA release in these brain regions[4]. The present finding is not only consistent with our previous findings, but also demonstrates directly that immobilization stress causes increases in NA release in anterior hypothalamic adrenergic neurons.

Our previous study investigating time-related NA turnover in rat brain regions under conditions of immobilization stress showed that the elevation of NA turnover caused by stress continued for 1 h during exposure to stress[5]. The present study further shows that elevation of NA release persisted until after release from stress and did not recover to basal levels until 2 h after release from stress. We reported earlier that MHPG-SO_4 is increased even 50 min after release from immobilization stress[12]. It is suggested that the present finding indicates directly the above phenomenon. The present study further supports our earlier findings that central NA neurons are activat-

ed under stressful situations and are involved in mediating the effects of stress.

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Developmental exposure to organic lead causes permanent hippocampal damage in Fischer-344 rats

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Summary. The long-term consequences of neonatal exposure to triethyl lead, the putative neurotoxic metabolite of the anti-knock gasoline additive tetraethyl lead, were examined with respect to central nervous system (CNS) development. We presently report a series of studies in which exposure of neonatal rats to organic lead produces profound CNS damage in adulthood as indicated by dose-dependent, persistent behavioral hyperreactivity as well as dose-dependent, preferential, and permanent damage to the hippocampus. General morphological parameters of brain development were not altered. Pharmacological probes of neurotransmitter system integrity suggested a functional and dose-dependent relationship between this behavioral hyperreactivity and hippocampal damage via cholinergic, but not dopaminergic, pathways. Furthermore, these alterations were not accompanied by long-term alterations in motor activity and were not attributable to the presence of lead in adult neural tissue. Finally, these behavioral, anatomical, and pharmacological indices of developmental exposure to organic lead were dissociable from any effects of early undernutrition. These data collectively indicate that organolead compounds may pose a potent neurotoxic threat to the developing CNS.
Key words. Triethyl lead; development; central nervous system; neurotoxicity; hyperreactivity; hippocampus; cholinergic; rat.

Organolead compounds were synthesized in the 1920's for use as gasoline anti-knock additives[1], and constitute the major sources of human lead exposure and environmental lead contamination[2]. Despite the recent decline in use of leaded gasoline additives in the U.S. (from 73% of total gasoline consumption in 1977 to 18% in 1988)[3], world-wide mine production of lead remains higher in the current decade than at any time in history, with the exception of the 1970's[4]. Of particular concern is the possibility that lead may be biotransformed into the more toxic organolead compounds[5,6]. Organic lead gasoline additives are highly lipid soluble, rapidly metabolized, and readily cross the blood-brain barrier[7,8]. These physiochemical properties make the central nervous system (CNS) a critical target organ for organolead toxicity.

With respect to potential health effects, it has been estimated that up to 20% of lead in the brains of urban dwellers is of the organic form[9]. Unlike the case with inorganic lead toxicity, however, routine blood and urine monitoring are of questionable values because of a relatively short residence time of organic lead in blood and a lack of correlation of organic lead levels in urine with CNS effects[10]. A lack of accumulation of organic lead in bone also renders this prognostic aid ineffective[10]. Therapeutic treatment of organolead poisoning, such as by chelating agents, is also problematic. Based on the fact that EDTA binds Pb^{2+} firmly, chelation therapy is often recommended; however, as it does not bind Et_3Pb^+ or Et_2Pb^{2+} it may not be very useful[11].

Adverse health effects of organolead toxicity may be further exacerbated for specific subpopulations. Indeed,