DMPP may be able to trigger, through different mechanisms, a poorly-characterized reflex starting from vascular chemo- and/or pressoreceptors which eventually restores tissue perfusion to a point compatible with survival. Such receptors are apparently greatly sensitized in conditions of hypovolemic shock, since they can be activated by the above drugs at doses that are almost ineffective under normal conditions.

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Catecholamine metabolism in the vas deferens and the adrenal gland with special reference to the central catecholamine-depleted state

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Abstract. Experiments were carried out to elucidate the role of central catecholamines in regulating catecholamine metabolism in the vas deferens and adrenal gland of the rat. Rats were injected intracerebroventricularly (i.c.v.) with either vehicle or 6-hydroxydopamine (6-OHDA). Groups of animals pretreated with vehicle or 6-OHDA (i.c.v.) were injected intraperitoneally (i.p.) with alpha-methyl-para-tyrosine (AMT), a tyrosine hydroxylase inhibitor. Catecholamine turnover rates were estimated by determining norepinephrine or epinephrine content after administrating AMT.

Central norepinephrine and dopamine contents decreased significantly ($p < 0.05$) after treatment with 6-OHDA and AMT. The norepinephrine content of the vas deferens of rats pretreated with 6-OHDA was markedly reduced ($p < 0.001$) after administration of AMT, whereas that of the vehicle-treated rats remained unchanged. Administration of 6-OHDA had no effect on the norepinephrine or epinephrine content of the adrenal gland.

The present results indicate that central monoaminergic neurons have an inhibitory effect on the adrenergic neurons of the vas deferens. In contrast, this inhibitory regulation does not appear to be exerted on the adrenal glands.
Key words. Catecholamine; vas deferens; adrenal gland; 6-hydroxydopamine; alpha-methyl-para-tyrosine.

The catecholamine content of peripheral organs varies widely depending on the density of innervation by adrenergic nerve fibers. The vas deferens is densely innervated and has a high norepinephrine content[1,2]. The adrenal gland produces a vast amount of catecholamines; the mechanism of catecholamine release by this organ is different from that of release by adrenergic nerve endings[3,4]. Although catecholamine release from both these organs is regulated by the central nervous system, they are probably regulated differently by central catecholaminergic mechanisms. The results of previous studies have revealed that the central catecholamine-depleted state causes an increase in the norepinephrine content of the adrenal glands of monkeys[5], but a decrease in the norepinephrine content of the adrenals of rats[6]. On the other hand, it has also been reported that the depletion of central catecholamines has no discernible effect on the concentration of adrenal catecholamines[7]. Thus, earlier findings on the relation between the depletion of central catecholamines and adrenal catecholamine metabolism are rather fragmentary and contradictory. Furthermore, the effect of central catecholamines on catecholamine metabolism in the vas deferens has not yet been studied. We investigated the effect of central catecholamine depletion induced by i.c.v. injection of 6-hydroxydopamine (6-OHDA) in combination with the peripheral administration of alpha-methyl-para-tyrosine (AMT), a tyrosine hydroxylase inhibitor, on the catecholamine metabolism of both organs.

Materials and methods

Male Wistar rats were transferred to our laboratory at 6 weeks of age. The rats were maintained under constant room temperature conditions of 22–24 °C and a 12-h illumination cycle, given access to rat chow (CE-II, Japan Clea Co.) and distilled tap water available ad libitum, and used in experiments at the end of 2 weeks.

Rats were randomly divided into two groups of approximately 80 animals and treated as follows: group I, intracerebroventricular injection (i.c.v.) of saline-ascorbic acid (vehicle); group II, 6-OHDA (250 µg/kg i.c.v.).

The systolic blood pressure of each rat was determined in the conscious state once a week by a modified indirect tail cuff method using a pulse pick up, and recorded on a USM-105 physiograph (Ueda Instrument Co., Tokyo, Japan)[8].

On day 3 or day 14 after i.c.v. injection, the rats in group I and group II were subdivided into two groups: group I-1 (vehicle), vehicle treatment (i.c.v.); group I-2 (vehicle + AMT), vehicle treatment plus intraperitoneal (i.p.) injection of AMT (250 mg/kg); group II-1 (6-OHDA), 6-OHDA treatment (i.c.v.); group II-2 (6-OHDA + AMT), 6-OHDA treatment plus i.p. injection of AMT (250 mg/kg).

Vehicle- and 6-OHDA-treated rats not injected with AMT (groups I-1 and II-1) were immediately decapitated. AMT-injected rats pretreated with vehicle or 6-OHDA (group I-2 or II-2) were decapitated 4 h after administration of AMT. Both adrenal glands, vasa deferentia and the whole brain were quickly removed.

Intracerebroventricular injection of 6-OHDA. 6-Hydroxydopamine hydrobromide was dissolved in a 0.9% saline solution containing ascorbic acid (1 mg/ml) as an antioxidant. Under pentobarbital anesthesia (30–40 mg/kg, i.p.), each rat was i.c.v. injected stereotaxically[9]. The volume injected was 20–25 µl.

Assay of norepinephrine, epinephrine and dopamine. Norepinephrine, epinephrine and dopamine content was estimated by high-performance liquid chromatography (HPLC) with electrochemical detection according to the method described by Wagner et al.[10] with a slight modification[11,12]. The adrenal gland and vas deferens were homogenized in 1 ml of 0.2 M perchloric acid containing 100 µM EDTA and an appropriate quantity of 3,4-dihydroxybenzylamine as an internal standard, and the whole brain was homogenized in 10 ml of the same solution. The homogenate was centrifuged at 10,000 × g for 20 min. at 4 °C. The supernatants were injected into a high-performance liquid chromatograph. The chromatographic mobile phase consisted of 0.1 M sodium acetate and 0.1 M citric acid buffer (pH 3.9) containing 15% methanol, 10 µM EDTA and 160 mg sodium 1-octanesulfonate, pumped at 1.0 ml/min. The potential of the electrochemical detector was set at 650 mV against the Ag/AgCl reference electrode. The working electrode was of glassy carbon.

HPLC. The HPLC system consisted of an Eicom DG-100 degasser, EP-10 pump system, SI-100 sample injector, ECD-100 electrochemical detector, and Eicompak MA-ODS column (4.6 × 250 mm) (EICOM CORP. Kyoto, Japan).

Estimation of rates of norepinephrine and epinephrine turnover. The rates of norepinephrine and epinephrine turnover were estimated by measuring the decrease in norepinephrine and epinephrine content after inhibiting tyrosine hydroxylase with AMT as described by Brodie et al.[13]. As reported by Miwa et al.[14,15], the content of norepinephrine in peripheral organs decreases exponentially up to 8 h after i.p. injection of AMT (250 mg/kg). In this experiment, we measured norepinephrine and epinephrine content 4 h after AMT administration.

Statistical analysis. All results are expressed as means ± standard deviation (mean ± SD). Statistical significance was determined by one-way analysis of variance and Dunnett's multiple comparisons test.

Results

6-OHDA had no effect on the rate of body weight gain or the ratio of the weight of whole brain and adrenal gland to total body weight. The systolic blood pressures of vehicle- and 6-OHDA-treated rats showed similar ten-

dencies over time, and there were no significant differences in blood pressure on any of the days examined.

Figure 1 shows the norepinephrine content of the whole brain of the vehicle- and 6-OHDA-treated groups and the effect of i.p. administration of AMT on both groups on day 3 and day 14 after the i.c.v injections. The norepinephrine content of the brains of the 6-OHDA-treated group on day 3 and day 14 were 59.8% ($p < 0.05$) and 63.1% ($p < 0.05$) of the corresponding levels in the vehicle-treated group ($221.0 \pm 36.9 : 369.8 \pm 87.9$ ng/g and $241.9 \pm 75.1 : 383.5 \pm 52.1$ ng/g). The norepinephrine content in both groups was significantly lower ($p < 0.05$) 4 h after systemic administration of AMT.

Figure 2 shows the dopamine content of the whole brain of vehicle- and 6-OHDA-treated rats. The effect of AMT at each time was also examined. Changes in dopamine content were similar to the changes in norepinephrine. The dopamine content of the brains of 6-OHDA-treated rats on day 3 and day 14 was 73.2% ($p < 0.05$) and 84.3% ($p < 0.05$) of the corresponding levels in the vehicle-treated rats ($680.6 \pm 96.5 : 929.8 \pm 99.5$ ng/g and $830.9 \pm 67.2 : 985.8 \pm 99.6$ ng/g), and there was a signif-

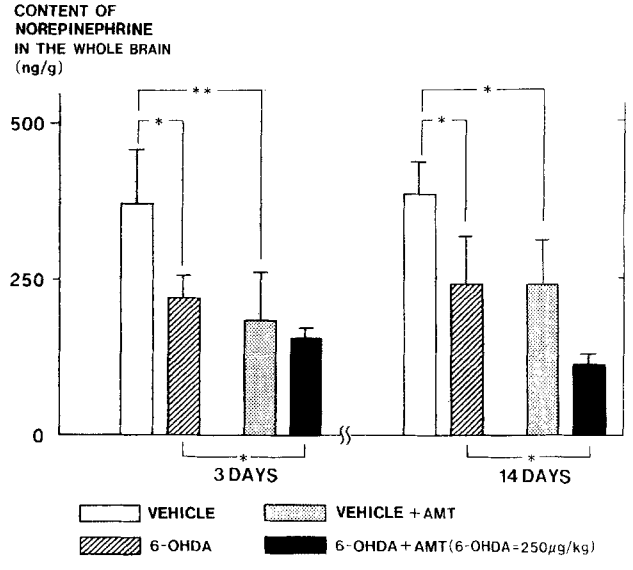

Figure 1. Whole brain norepinephrine content of rats injected i.c.v. with vehicle (vehicle), i.c.v. with 6-OHDA (6-OHDA), i.c.v. with vehicle plus i.p. AMT (vehicle + AMT) or i.c.v. with 6-OHDA plus i.p. AMT (6-OHDA + AMT). Abscissa: days after the injections. Each bar represents a mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001.

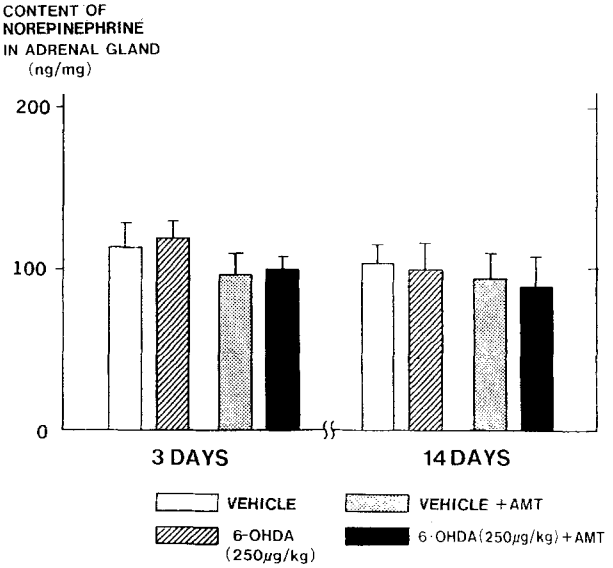

Figure 3. Adrenal gland norepinephrine content of rats treated as described in fig. 1. Explanations are the same as in fig. 1.

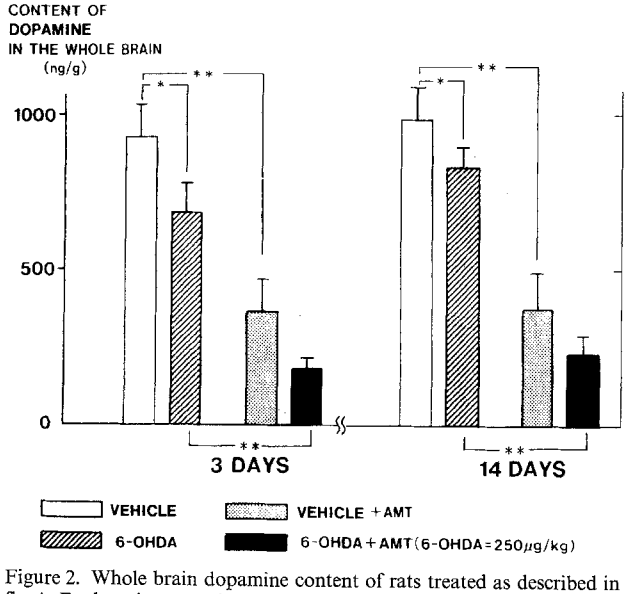

Figure 2. Whole brain dopamine content of rats treated as described in fig. 1. Explanations are the same as in fig. 1.

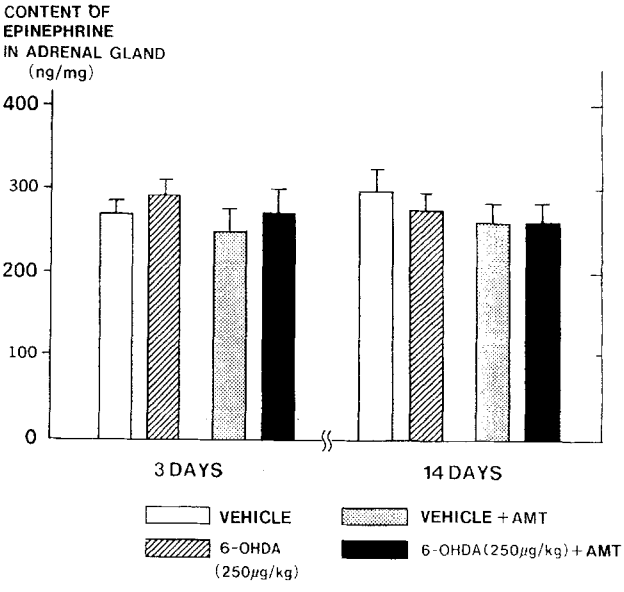

Figure 4. Adrenal gland epinephrine content of rats treated as described in fig. 1. Explanations are the same as in fig. 1.

icant decrease in dopamine content in both groups after AMT administration.

Figure 3 shows the norepinephrine content of the adrenal glands of vehicle- and 6-OHDA-treated rats in the presence and absence of i.p. injection of AMT on day 3 and day 14 after their i.c.v. injections. There was no significant difference in the norepinephrine content of the adrenals of the vehicle- or 6-OHDA-treated rats at either time. The norepinephrine content of the vehicle- or 6-OHDA-treated rats remained unchanged after AMT administration.

Figure 4 shows the epinephrine content of the adrenal glands of vehicle- and 6-OHDA-treated rats in the presence and absence of AMT administration. There was no significant difference between the epinephrine content of the adrenals of vehicle- and 6-OHDA-treated rats.

Figure 5 shows the norepinephrine content of the vas deferens of vehicle- and 6-OHDA-treated rats in the presence and absence of i.p. administration of AMT on day 3 and day 14 after the i.c.v. injections. There was no significant difference between the norepinephrine content of the vasa deferentia of vehicle- and 6-OHDA-treated rats. On day 3 after the i.c.v. injections, the norepinephrine content of the 6-OHDA-treated rats was significantly lower after AMT administration (from 9.22 ± 0.47 to 7.86 ± 0.64 ng/mg) ($p < 0.001$), whereas that of vehicle-treated rats remained unchanged after AMT administration (from 9.94 ± 1.02 to 9.58 ± 0.45 ng/mg). Subsequently, on day 14 after the i.c.v. injections, the norepinephrine content of 6-OHDA-treated rats was significantly lower after AMT administration (from 9.75 ± 1.04 to 7.43 ± 2.20 ng/mg) ($p < 0.05$), whereas levels in the vehicle-treated rats decreased only slightly (from 9.82 ± 1.38 to 8.99 ± 1.06 ng/mg).

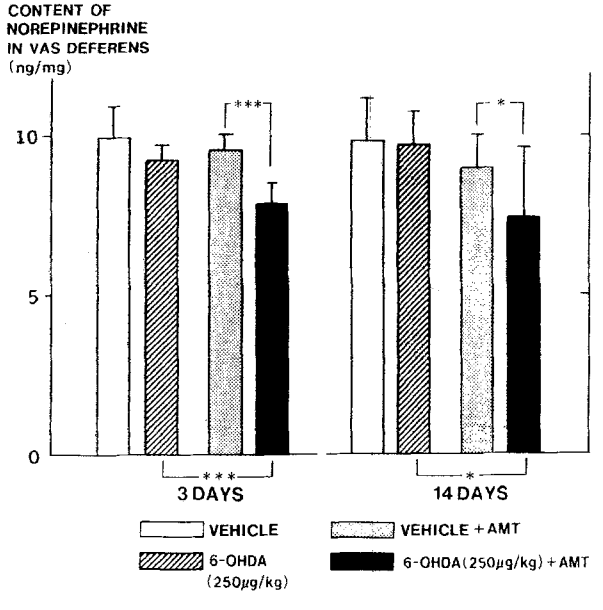

Figure 5. Norepinephrine content of the vas deferens of rats treated as described in fig. 1. Explanations are the same as in fig. 1.

Discussion

The results of the present study are consistent with earlier findings that injection of 6-OHDA into the cerebrospinal fluid of the rat leads to a decrease in brain catecholamine content[16–18]. AMT also reduced brain catecholamines in rats whether treated with i.c.v. 6-OHDA or not. The reduction in brain catecholamines by AMT or 6-OHDA alone did not alter the catecholamine content of peripheral organs.

Histochemical studies have demonstrated the presence of adrenergic and cholinergic nerve fibers in the vas deferens[19]. It is generally accepted that adrenergic nerve fibers in the vas deferens play an important role in the contraction of this organ and are probably responsible for the seminal emission[16]. Some central noradrenergic nerves originating in the pons terminate in the autonomic lateral cell columns of the thoracic and sacral spinal cord[20,21]. The efferent neurons arising in the thoracolumbar ejaculatory center of the spinal cord course through the hypogastric nerves to the vas deferens[22]. It would therefore appear reasonable that these systems play a significant role in regulating autonomic activity in the vas deferens. After AMT administration the rate of norepinephrine depletion in the vas deferens of the 6-OHDA-treated rats was greater than in the vehicle-treated rats (fig. 5). Hence, the rate of norepinephrine turnover in this organs was probably higher in the 6-OHDA-treated rats. These findings imply that under physiological conditions central monoaminergic neurons (namely the descending noradrenergic neurons) exert an inhibitory effect on adrenergic neurons in the vas deferens via the ejaculatory center of the thoracolumbar spinal cord.

Cells in the adrenal medulla are innervated by preganglionic acetylcholine-containing sympathetic nerve fibers which arise in the anterior spinal nerve roots of T7 to L3 and join the greater and lesser splanchnic nerves[23]. According to earlier studies, stimulation of the base of the 4th ventricle of the medulla oblongata or ventromedial hypothalamic nucleus results in enhancement of adrenomedullary function, and the spinal cord plays an important role as the secondary center of adrenomedullary activity[24,25].

In addition, central norepinephrine regulates the secretion of pituitary hormones. In experimental animals, the release of central norepinephrine results in a decrease in the secretion of ACTH and subsequent reduction of cortisol production[26]. Since epinephrine synthesis in the adrenal medulla is controlled by cortisol[27], it appears that central norepinephrine has some effect on adrenomedullary catecholamine secretion.

Gauthier and Reader reported that i.c.v. injection of 6-OHDA depletes adrenal catecholamines[6]; however, there is a diversity of opinion as to the exact sequence of events. An increase in the norepinephrine content of the adrenal glands[5] and an elevation of adrenal tyrosine hydroxylase activity[28] as a result of the central action of 6-OHDA have also been reported. Our results indicate

that neither the epinephrine nor the norepinephrine content of the adrenal glands changed after central 6-OHDA administration. Further studies will be required to obtain more detailed information concerning the role of central catecholamines in the metabolism of adrenal catecholamines.

In summary, the present results indicate that under physiological conditions, central monoaminergic neurons have an inhibitory effect on the adrenergic neurons of the vas deferens. This inhibitory regulation, however, does not appear to be exerted on the adrenal glands. The roles of the central catecholamines in the regulation of catecholamine metabolism in the adrenal glands remain to be elucidated in further studies.

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Rapid isolation of mitochondrial DNA. Mitochondrial DNA from *Drosophila serrata*

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Abstract. A simple and rapid method for isolation of high quality mitochondrial DNA (mtDNA) is presented in this report. Using this method, isolation and restriction site maps for 10 enzymes of the mtDNA of *Drosophila serrata* were established.
Key words. Mitochondrial DNA; isolation; *Drosophila serrata.*

Analysis of mtDNA has proved to be a powerful tool in many fields of biology. Simple and rapid methods for mtDNA isolation are needed especially in population genetics where many samples have to be examined. High quality mtDNA is essential, so that digestion enzyme analyses and Southern blot hybridizations yield unequivocal results. We describe here a rapid and efficient two-step method of mtDNA isolation: a) the purification of the mitochondria, avoiding the nuclear DNA, and b) the isolation of the mtDNA. The reagents used in this protocol are very common in every laboratory [1].

Using the above method, the mtDNA of *Drosophila serrata*, a species belonging to the *montium* subgroup of the *melanogaster* species group [2, 3], was isolated and digested with several restriction enzymes. Both restriction enzyme analyses, and hybridizations mtDNA fragments either from *D. serrata* or from *D. melanogaster* as probes, result in a detailed map for 10 restriction endonucleases.

Materials and methods

Animals. Drosophila serrata, strain No. 3018.1 from the University of Texas Stock Center [3] and *D. melanogaster,* Canton/S (C/S) were used in the present study.

Isolation of the mtDNA. The method used to isolate the *Drosophila* mtDNA, is described below: