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Implication of Cerebral Dopamine-β Hydroxylase for Cardiovascular and Mood Regulation in Rats

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Abstract

The essentiality of the role of norepinephrine (NE) in the central nervous system has recently been reconsidered. NE exerts many effects and mediates a number of functions in living organisms. Dopamine-β-hydroxylase (DBH) is the crucial enzyme for NE and epinephrine biosynthesis. Removal of this enzyme causes deficient NE at sympathetic terminals characterized by orthostatic hypotension in humans. The hypothesis tested in this study was that NE deficiency in the central nervous system caused autonomic failure in cardiovascular regulation. The immunotoxin anti-DBH-saporin (DSAP) was used to examine the putative role of cerebral NE. Male Sprague-Dawley rats were injected, intracerebroventricularly (icv), with DSAP and cardiovascular reactivity, as well as behavioral variables in the open-field locomotion test (OLT), sucrose intake test (SIT) and forced swim test (FST), were monitored for changes. The results indicated that treatment with DSAP caused significant reductions in spontaneous blood pressure (BP) and heart rate (HR), and a decrease in the rearing position on the OLT, in the same group of rats. In addition, a significant increase in mobility with low concurrent immobility frequencies was observed on the FST. However, there was no variation on the SIT. In conclusion, a deficiency in the cerebral DBH might dysregulate the autonomic outflows and, thus, leads to lower BP and HR. However, there was no mood change such as despair or anhedonia observed in the experiments.

Key Words: cardiovascular, cerebral, dopamine-β-hydroxylase, mood

Introduction

Catecholamine molecules are crucial neurotransmitters in both the central and peripheral nervous systems (10, 37). In the central nervous system, norepinephrine (NE) and putative epinephrine are localized to several neuronal populations in the hindbrain and midbrain. Ascending fibers affected by NE, from the various cell groups, project mainly through the dorsal and ventral bundles to the forebrain regions such as the frontal cortex, hippocampus, amygdale and hypothalamus (15). These sites, along with the convergence of cerebral sympathetic pathways, mediate many physiological effects, including emotion, anxiety and the regulation of the central autonomic outflows (1, 10, 20, 37). In the periphery, the sympathetic nervous system exerts widespread control from cardiovascular regulation to energy balance (10, 35).

Numerous physiologically and emotionally motivated behaviors are characterized by complimentary and coordinated control of motor and autonomic outflows (32, 35). One of the major achievements in the understanding of cardiovascular regulation has been the recognition of the intimate interaction between the central nervous system and the cardiovas-

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cular system, largely mediated by autonomic regulation (6, 12, 35). The effects of the release of peripheral NE cannot be over emphasized in the evolution of human beings; peripheral NE exerts its effects on the cardiovascular system to facilitate and maintain an upright posture. Accordingly, orthostatic blood pressure (BP) has to be steadily maintained using many regulatory mechanisms, such as suprabulbar mechanisms which are thought to be involved but are not fully understood (12, 16, 31, 32, 44).

Dopamine-β-hydroxylase (DBH) is the key enzyme that leads to NE synthesis; it is critical for the NE levels in synaptic neurotransmission. The biochemical features, autonomic physiology and physical symptoms associated with human DBH deficiency have been well documented (7, 26). DBH-deficient patients exhibit the well-known symptom, orthostatic hypotension, characterized by complete absence of NE in postganglionic neurons, which exerts tonic sympathetic-outflow activation (SOA) in the periphery critical for the NE levels in synaptic neurotransmission (3, 7, 25, 41). Dysregulation of cerebral NE may also be involved in the pathophysiology of orthostatic hypotension (8, 25, 41); however, this has not been confirmed. There is no animal model to confirm this possibility.

In the present study, cerebral noradrenergic neurons were studied to determine whether they could maintain the basal SOA in conscious rats after depletion of DBH. Cerebral DBH-deficiency rats were developed after intracerebroventricular (icv) microinjection of an anti-DBH antibody conjugated to the ribosomal toxin saporin (DSAP). Measurements were carried out, and measurements together with cardiovascular reactivity and behavioral paradigms were also studied (locomotor activity, hedonics and despair, *i.e.* open-field locomotion, sucrose preference and the forced swim test).

Materials and Methods

Animals

Adult male Sprague-Dawley (SD) rats (BioLASCO, Taipei, Taiwan, R.O.C.), weighing between 250 and 300 g, were used. All rats were housed in a temperature- and humidity-controlled holding facility with 12 h light/dark cycles (light on from 07:00 to 19:00) maintained by manual light control switches as required by the experiment. Rats in the same experimental group were housed in the same cage. All rats received food and water *ad labitum*. The experiments were performed between 08:30 and 21:00, with all rats being tested at the same time everyday whenever possible. All experimental procedures were evaluated and approved by the Animal Care Committee of the National Defense Medical Center. All efforts were made to keep the number of animals used as low as possible and to minimize animal suffering during the experiments.

General Procedures

A total of 32 rats were assigned randomly into two experiments evenly selected according to bodyweight. In each experiment, the rats were subdivided into two groups (n = 8 each); one group was a control group used for comparison of DBH depletion; the other was the study group where the effects of depleted DBH were studied. For DBH depletion, the rats were microinfused, into the left icv, with the toxin anti-DBH-saporin (DSAP, 10 μg/10 μl) (Advanced Targeting Systems, SanDiego, CA, USA) under pentobarbital anesthesia using stereotactic coordinates (1.2 mm anterior to bregma, 1.0 mm lateral to the midline, and 4.5 mm beneath the dural surface). For the control rats, the same volume of vehicle (free saporin) was microinfused into the left icv. Animals were closely monitored daily during the treatment with toxin for overt signs of stress by examination of their general behavior, including food and water intake.

All experiments were performed in the same quiet isolated room to minimize external influences on the hemodynamic measurements. In Experiment 1, multiple sequential tests were performed with each rat as defined below. The rats participated in sequential tests, including the open-field locomotion test (OLT) in the morning, and tail-cuff measurements of BP and heart rate (HR) in the afternoon; the sucrose intake test (SIT) was carried out in the evening. In Experiment 2, another group of rats, which were of the same age as those used in Experiment 1, was used only to study the FST during the daytime in a darkroom. After the final session of each experiment, the rats were sacrificed and their brains collected for ELISA and immunofluorescence staining.

Behavioral Testing

Open-Field Locomotion Test (OLT)

OLT was conducted by using a computerized, automated activity monitoring system (MED Associates, Inc., St. Albans, VT, USA). This system included four novel plexiglass chambers ($43.2 \times 43.2 \times 30.2$ cm^3) equipped with two horizontal photobeamarrays; the lower horizontal array had 16-photo beams on each side and the upper array had 16-photo beams on opposite sides set at 4.75 cm above the lower row. There were corresponding light sources that emitted photobeams 3 cm apart and 4.5 cm above

the chamber floor. The test was identical to the procedure used by the same team in a previous study (34). The experiment began with three consecutive sessions for the animals to habituate to the chamber. The test was then performed and data were collected before and after the DSAP administration (day 0) on day -1 and day 14 between 08:30 a.m. and 11:30 p.m. In brief, the rats were individually placed into these chambers with the lights off for a 1-h session. During this session, there was an adaptation period for 30 min without data collection; in the second 30-min period, different activity counts were summed and recorded by a programmed microcomputer. The focus was on the following behaviors that were measured with the help of an activity monitor.

[1] Ambulatory count (number): Ambulation is the basic characteristic of global animal horizontal activity used in open field studies. Horizontal activity was measured as the number of consecutive photobeam breaks.
[2] Stereotypic count (number): Scratching, grooming and head swings during which the animals remain in the same location but interrupt the photobeams. The number on these activities was recorded and defined as the stereotypic count.
[3] Rearing count (number): Rearing count was defined as the number of standing positions on hind limbs with uplifted forelimbs vertically by the animals.

Sucrose Intake Test (SIT)

SIT was carried out during the light-off phase, which lasted 3 h (between 17:00 and 20:00) on the same day of the locomotion test. The rats were placed into single cages 2 h before the testing and housed in groups again at the end of the test. Two bottles were used, one filled with 1% sucrose solution and the other with water. The rats continued to have free food access throughout each test. Sucrose and water consumption were measured for 1 h by weighing the bottles both at the beginning and the end of the test. The sucrose preference was measured by calculating the proportion of sucrose solution consumption out of the total consumption of water and sucrose solution.

Forced-Swim Test (FST)

The FST apparatus used in this study was similar to that previously described by Cryan *et al*. (13, 14). It consisted of two home-made pyrex cylinders with a diameter of 25 cm and a height of 50 cm (Noldus Information Technology, Wageningen, The Netherlands). Each cylinder was filled with water to a depth of 30 cm for the experiment; the temperature was set at approximately 25°C. In brief, before the test, the rats were individually placed into these cylinders for 15 min for adaptation. After the adaptation, the rats were removed and dried before being returned to their home cages. These rats were again placed in the cylinders 24 h later to start the test. The behavioral session (between 09:00 and 17:00) was carried out before and after DSAP administration (day 0) on day -6, day -4 and day -2 (baseline value) and day 7, day 14, day 21 and day 28. All behaviors were monitored from above by video camera *via* video tracking software for automated behavioral data analysis (EthoVision XT, Noldus Inormation Technology, The Netherlands) in the darkroom with a fixed background lighting setup. A time sampling technique was employed whereby a discrete variable with two possible behavioral states, mobile and immobile in each 5-sec time bin of the 300-sec test was recorded. The mobility state is established for each test according to the value of swim average activity (swimming and climbing) relative to the thresholds. The immobility state was assigned when no additional activity was observed other than that required to keep the rat's head above the water. The potency of mobility and immobility was measured by calculating the proportion of their frequency out of the mean of three baseline values before DSAP administration.

Tail-Cuff Methods for Hemodynamic Measurements

The measurements were performed between 13:00 and 16:00 on the same day after the OLT, with lights off to avoid the effects of illumination. The Columbus instruments' non-invasive BP (NIBP-8) monitor, with a warming compartment, was used in this study to examine systolic, diastolic, mean blood pressure (SBP, DBP, MBP, respectively) as well as HR; the measurements were taken for 16 sec for each rat. Subsequent measurements were set 30-sec apart. Simultaneous values of SBP, DBP, MBP and HR were obtained by estimating the average reading of three trial measurements.

Biochemical Studies

Norepinephrine Assay

The brains were sliced into sixteen 35 μm thick slices in the coronal plane. Four different brain regions from the prefrontal cortex (PFC), bed nucleus of the stria terminalis (BNST), locus coeruleus (LC) and A1 area were dissected following standard procedures (4). Sample tissues were homogenized and the supernatant was analyzed by enzyme-linked

immunosorbent assay for NE according to the manufacturer's instructions (DIAsourceImmunoAssay Diagnostics, Louvain-La-Neuve, Belgium).

Norepinephrine-Containing Neurons Staining

The presence of tyrosine hydroxylase (TH) and DBH immunoreactive cells, *i.e.* NE-containing neurons, were evaluated at the hindbrain LC and A1 sections and processed for double immunofluorescence labeling with the following markers: TH and DBH. Tissue preparation and immunohistochemistry procedures were performed as reported previously (17). Briefly, the cryoprotected rat brain was frozen; 35-μm coronal sections through the frontal cortex were cut with a cryostat. All immunohistochemistry was carried out on free-floating sections. The sections were pre-blocked with 10% normal rabbit serum (NRS) and 0.1% Triton X-100 in 0.1 M PBS (TBS) and incubated with either a mouse anti-TH monoclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or a mouse anti-DBH monoclonal antibody (Santa Cruz) as the primary antibodies. The TH antibody was used at 1:1000, and the DBH antiserum was used at 1:500 dilutions. Affinity-purified rabbit anti-mouse IgG (1:500) (Sigma Chemical Co., St Louis, MO., USA) was used as the secondary antibody. The Chemiluminescent HRP substrate Kit (Millipore, Temecula, CA., USA) was used to visualize the immunoreaction. Control experiments were processed in parallel. Selected double-stained sections were further processed for images acquired with an inverted fluorescent microscope equipped with a digital camera.

Statistical Analyses

The data were analyzed using SPSS (version 12.0, IBM, Chicago, IL, USA) for the variables of interest. For all behavioral tests, a two-way analysis of variance (ANOVA) was conducted with the between-subject factor of treatment (control or icv-DSAP) and within-subject factor of repeated measurement (time block). Further analyses and *post-hoc* multiple comparisons were performed with the Least Significant Difference (LSD) test where appropriate. The independent *t*-test was used to determine between-group differences at a given point in time and to compare the control and icv-DSAP treatment rats. All data are presented as means ± SEM. P value < 0.05 was considered statistically significant.

Results

Experiment 1: Effect of DBH Depletion on OLT, Hemodynamic Magnitude and SIT

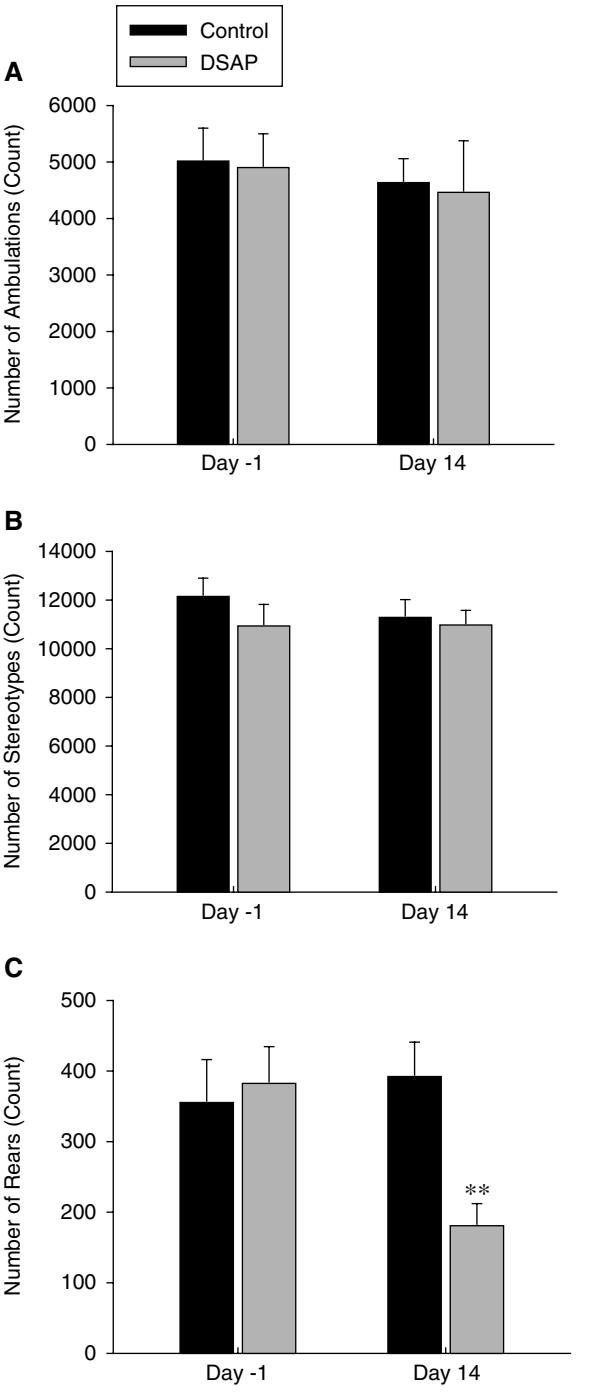

Fig. 1. Locomotor responses the day before (Day -1) and 14 days after (Day 14) the icv treatment with a control vehicle (free saporin) or DSAP (anti-DBH/saporin) on (A) horizontal activity (Ambulation), (B) stereotyped movement (Stereotype), and (C) vertical positions (Rearing). Each bar is expressed as the means ± SEM **$P < 0.01$ indicates a significant difference compared to the corresponding vehicle control data.

After the 14-day administration of DSAP (Fig. 1), DBH depletion did not affect either ambulation (horizontal activity, Fig. 1A) or stereotypic behavior

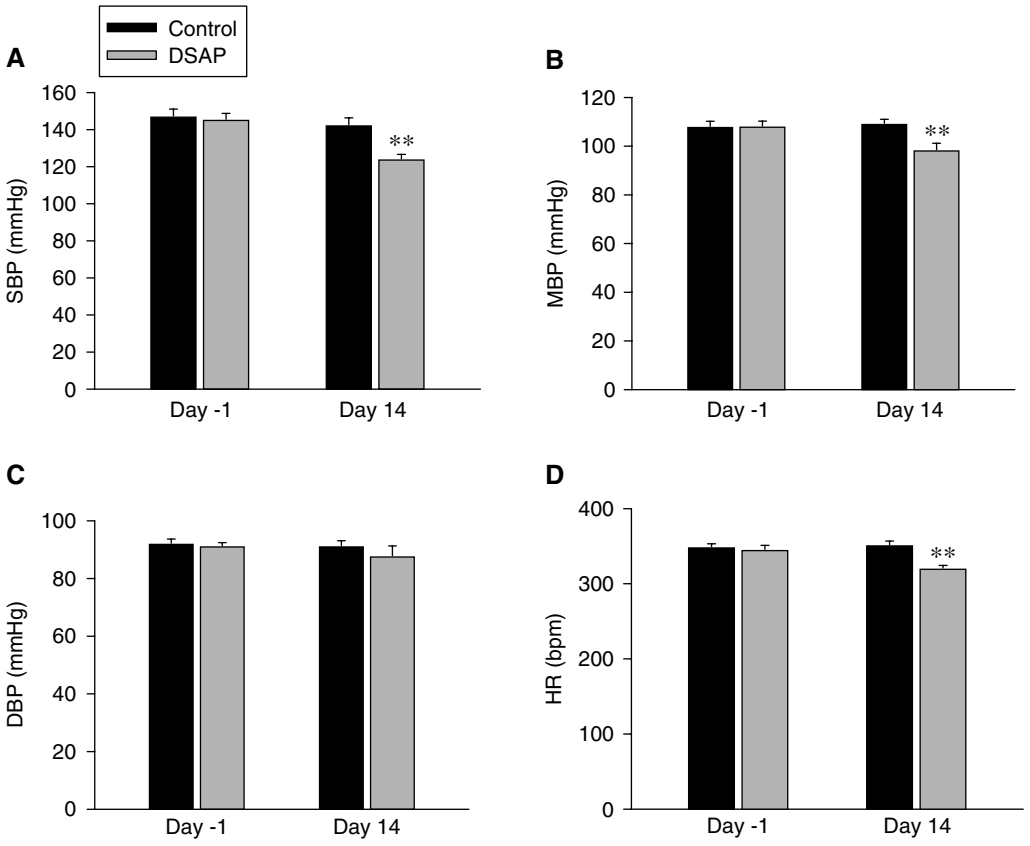

Fig. 2. Cardiovascular responses on the day before (Day -1) and 14 days after (Day 14) the icv treatment with control vehicle (free saporin) or DSAP (anti-DBH/saporin) on (A) systolic blood pressure (SBP), (B) mean blood pressure (MBP), (C) diastolic blood pressure (DBP), and (D) heart rate (HR). Each bar is expressed as the means ± SEM. $^{**}P < 0.01$ indicates a significant difference compared with the corresponding vehicle control data.

(stereotypic movements, Fig. 1B); however, the animal rearing (vertically position, Fig. 1C) decreased. For the former two behaviors, there was no significant change after DSAP treatment (ambulatory count: $F(1,14) = 0.038$, $P = 0.849$; stereotypic count: $F(1,14) = 1.026$, $P = 0.328$), effect of time-block (day -1 *vs.* day 14) repeated measurement (ambulatory count: $F(1,14) = 0.690$, $P = 0.420$; stereotypic count: $F(1,14) = 0.440$, $P = 0.518$), and time-block × treatment interaction (ambulatory count: $F(1,14) = 0.003$, $P = 0.955$; stereotypic count: $F(1,14) = 0.531$, $P = 0.478$). However, for the rearing behavior, there were no significant changes after DSAP treatment ($F(1,14) = 2.554$, $P = 0.132$) except for the main effects of the time-block (day -1 *vs.* day 14) repeated measurement ($F(1,14) = 5.856$, $P = 0.030$) and the time-block × treatment interaction ($F(1,14) = 12.272$, $P = 0.004$). The simple main-effect analyses followed by *post-hoc* multiple comparisons revealed that the DSAP treated rats had significant decrease in behavior on day 14 after treatment compared to day -1 before treatment ($F(1,7) = 10.478$, $P = 0.014$). Furthermore, the DSAP treated rats had significantly lower rearing scores on day 14 after DSAP treatment ($t(14) = 3.846$, $P = 0.002$) compared to the control rats.

As shown in Fig. 2, the effect of DSAP treatment on the cardiovascular responses of rats during the actual tail-cuff measurement trials was quite evident. There were no differences in the DBP ($F(1;14) = 0.830$, $P = 0.378$) between the DSAP-treated rats and the control rats (Fig. 2C); however, the SBP (Fig. 2A) and MBP (Fig. 2B) as well as the HR (Fig. 2D) were lower in the DSAP treated rats compared to the control rats. The two-way ANOVA confirmed significant DSAP treatment (SBP: $F(1,14) = 6.769$, $P = 0.021$; MBP: $F(1,14) = 4.795$, $P = 0.046$; HR: $F(1,14) = 14.561$, $P = 0.002$), effects with regard to the time-block (day 1 *vs.* day 14) repeated measurements (SBP: $F(1,14) = 24.571$, $P = 0.001$; MBP: $F(1,14) = 5.565$, $P = 0.033$; HR: $F(1,14)=7.313$, $P = 0.017$) and the time-block × treatment interaction (SBP: $F(1,14) = 9.951$, $P = 0.007$; MBP: $F(1,14) = 9.320$, $P = 0.09$; HR: $F(1,14) = 11.401$, $P = 0.005$) effects. Simple main effect analyses followed by *post-hoc* multiple comparisons revealed that the DSAP treated rats had significantly lower SBP ($F(1,7) = 21.833$, $P = 0.002$)

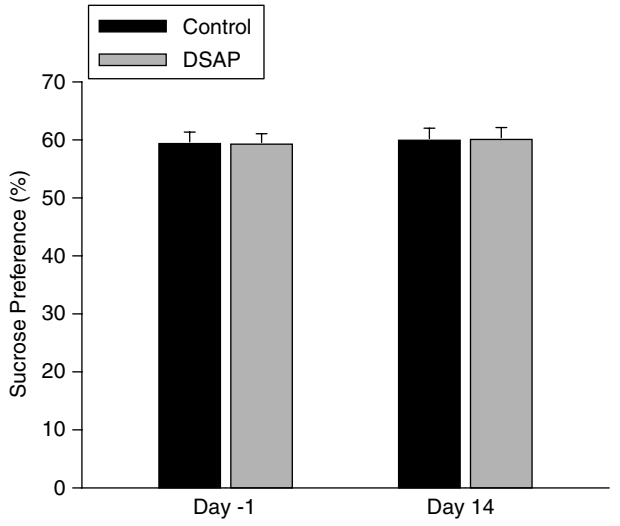

Fig. 3. Sucrose-intake responses on the day before (Day -1) and 14 days after (Day 14) the icv treatment with control vehicle (free saporin) or DSAP (anti-DBH/saporin); each bar is expressed as the means ± SEM.

and MBP (F(1,7) = 8.324, $P = 0.023$) as well as HR (F(1,7) = 10.422, $P = 0.014$) on day14 after treatment compared to day -1 before treatment. Furthermore, the DSAP treated rats had significantly lower SBP (t(14) = 4.099, $P = 0.001$) and MBP (t(14) = 3.446, $P = 0.004$) as well as in HR (t(14) = 5.394, $P = 0.001$) on day 14 after DSAP treatment compared to the control rats.

On the other hand, the sucrose preference in the SIT (Fig. 3) showed that there were no significant differences between the DSAP-treated rats and the control rats (F(1;14) = 0.001, $P = 0.996$); in addition, there were no significant differences between these two groups with regard to the time-block (day -1 *vs.* day 14) repeated measurements (F(1;14) = 1.936, $P = 0.186$).

Experiment 2: Effect of DBH Depletion on FST

DSAP and vehicle control treatments resulted in different patterns of FST behavior (mobility in Fig. 4A or immobility in Fig. 4B) on the days before and after DSAP treatment (day -6, day -4, day -2, day 7, day 14, day 21and day 28). As shown in Fig. 4, the two-way ANOVA revealed significant effects of DSAP treatment on mobility (F(1,14) = 6.388; $P = 0.024$) but not on immobility: F(1,14) = 2.220; $P = 0.158$); in addition, there were significant effects on the time-block repeated measurements for both mobility and immobility (mobility: F(6,84) = 9.299, $P = 0.000$; immobility: F(1,14) = 7.605, $P = 0.000$) as well as a time-block × DSAP interaction (mobility: F(1,14) = 3.709, $P = 0.003$; immobility: F(6,84) = 3.137, $P = 0.008$).

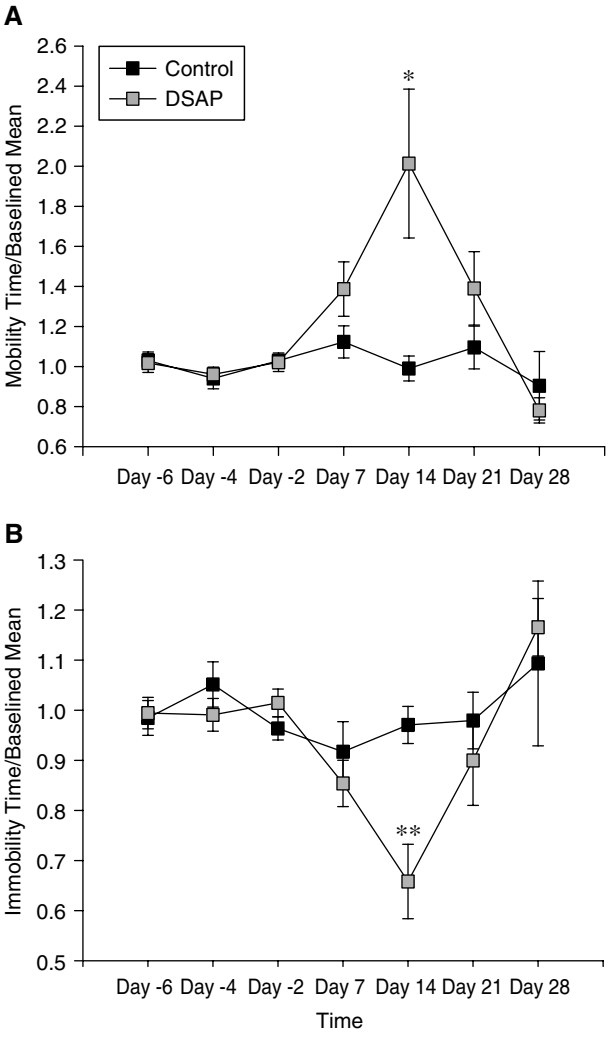

Fig. 4. Effects of anti-DBH/saporin-induced lesions on rat behavior on the forced swimming test: values represent counts of (A) mobility and (B) immobility behavior from six days before (Day -6) to twenty-eight days after (Day 28) icv-treated with control vehicle (free saporin) or DSAP (anti-DBH/saporin). Each square is expressed as the means ± SEM; *$P < 0.05$ and **$P < 0.01$ indicate a significant difference compared to the corresponding vehicle control data.

Simple main-effect analyses followed by *post-hoc* multiple comparisons revealed that the DSAP treated rats had a significant increase in mobility behavior on day 14 compared to day -6 ($P = 0.032$), day -4 ($P = 0.021$), day -2 ($P = 0.043$) and day 28 ($P = 0.019$). Similar effects were found between day -4 and day 28 ($P = 0.011$) as well as day -6 and day 28 ($P = 0.017$). Furthermore, the DSAP-treated rats had significantly greater mobility behavior on day 14 after DSAP treatment (t(14) = 2.715, $P = 0.017$) compared to the control rats.

By contrast, the DSAP-treated rats showed

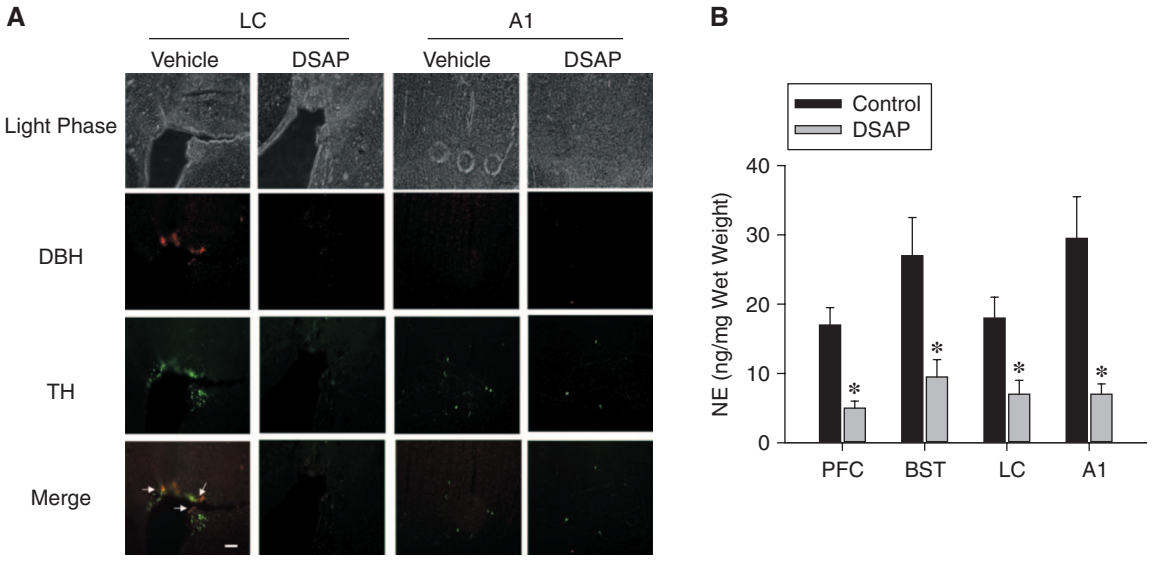

Fig. 5. Degeneration of cerebral noradrenergic neurons at 14 days after icv treatment with anti-DBH/saporin in rats: (A) Photomicrographs of neurons containing tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DBH) in the locus coeruleus (LC; left two panels) and A1 area (right two panels) of the hindbrain sections after control vehicle (free saporin) or DSAP (anti-DBH/saporin) treatment. The same frames are photographed with filter combinations for visualizing TH (green) and DBH (red) fluorescence. The bottom column is a representative photomicrograph of catecholaminergic neurons double-labeled for TH and DBH. Arrows indicate cell bodies labeled with both TH and DBH. Scale bars for pictures: 15 μm. (B) Norepinephrine (NE) levels in brain parts after control vehicle (free saporin) or DSAP (anti-DBH/saporin) treatment. Each bar is expressed as the means ± SEM; $*P < 0.05$ and $**P < 0.01$ for difference between groups.

significant decrease in immobility on day 14 compared to day -6 ($P = 0.009$), day -4 ($P = 0.001$), day -2 ($P = 0.003$), day 7 ($P = 0.001$), day 21 ($P = 0.031$) and day 28 ($P = 0.000$). Similar effects were also noted between day -4 and day 7 ($P = 0.015$), day -2 and day 28 ($P = 0.001$), day -2 and day 7 ($P = 0.044$), day -2 and day 7 ($P = 0.044$), day -2 and day 28 ($P = 0.017$), day 7 and day 28 ($P = 0.001$) as well as day 21 and day 28 ($P = 0.006$). Furthermore, the DSAP-treated rats showed significantly lower immobility on day 14 after DSAP treatment ($t(14) = 3.768$, $P = 0.002$) compared to the control rats.

Biochemical Studies: ELISA and Immunofluorescence Staining

After 14 days of DSAP treatment, the double-labeling TH/DBH immunofluorescence, at the cellular level, demonstrated that the hindbrain, particularly the LC-containing NE neurons, showed arrest of transmission after icv-exposure to DSAP (Fig. 5A). Moreover, ELISA showed that the NE levels were significantly reduced in the PFC ($t(24) = 2.228$, $P = 0.036$), BNST ($t(24) = 2.567$, $P = 0.017$), LC ($t(24) = 2.063$, $P = 0.050$) and the A1 area ($t(24) = 2.771$, $P = 0.011$) in the DSAP-treated rats compared to the control rats (Fig. 5B). These results provide evidence that DBH was successfully depleted throughout the selected brain regions *via* icv exposure of DSAP in the present experiments.

Discussion

An integrated central somatomotor-sympathetic system combining efferent pathways is acknowledged as important in maintaining homeostatic cardiovascular regulation and emotionally-motivated behaviors (12, 16, 31, 32, 44). Dysregulation in the system, possibly through cerebral noradrenergic neurons, leads to cardiovascular and affective disorders. Using three distinct behavioral paradigms for assessments, the experiments in this study showed that the toxin DSAP arrested cerebral noradrenergic neurons in rats and could differentially affect a variety of behaviors. The neuropathic rats exhibited less of a tendency to stand, rearing in the OLT (Fig. 1), and a significant reduction in cardiovascular reactivity (Fig. 2). However, in the assessments of depression, results showed no loss of interest (anhedonia) and hopelessness in the SIT (Fig. 3) and FST (Fig. 4).

The NE system innervates many different brain regions, such as the PFC, BNST, brainstem LC and A1 areas; these regions are known to play a significant role in the regulation of locomotor activity and emotionally motivated behaviors (38). These areas were affected by NE depletion after DSAP treatment in the present study. Histobiochemical studies confirmed that the neuropathic rats displayed massive

lesions of the ascending sympathetic projections by the observation of reduced TH/DBH immunoreactivity (Fig. 5A) and regional NE levels (Fig. 5B). There was a significant depletion of NE in all regions of the brain ranging from 75% in the A1 area to 68% in the PFC.

Effect of DBH Depletion on Locomotor Activity and Cardiovascular Correlation

Although patients with Parkinson's disease (PD) are commonly known to have complex symptoms associated with orthostatic hypotension (25), the state-dependent correlation of cerebral NE levels, locomotor activity and cardiovascular disturbances, associated with this syndrome, have not been determined. It is well known that the response to novelty, a vital function in maintaining vasomotor tonicity, depends largely on the SOA from the rostral portion of ventrolateral medulla (RVLM) (12, 16, 28). By contrast, in response to stress, an appropriate cardiovascular adjustment is depended upon synaptic relay in the RVLM from specific suprabulbar areas (12, 16, 31, 32, 44). In humans, DBH deficiency is characterized by orthostatic hypotension, with symptoms of orthostatic stress due to postganglionic neurons lacking the release of NE properly in response to rapid changes from the supine to standing positions (7, 25, 41). In rats, however, since they did not develop upright posture, like humans, detailed analysis of orthostatic hypotension did not apply. Therefore, changes in the rats were studied by determining the possible mechanisms correlated with locomotion and cardiovascular reactivity with a deficiency in cerebral DBH in the present study.

Delini-Stula *et al*. previously reported the use of DSP-4 treatment in rats exposed to a novel environment; suppression of locomotor exploratory responses, including ambulation, rearing and object approach, was noted (18). Such effects have not been consistently observed in other studies (2, 33). In addition, another approach used gene-knockout, which showed that exploratory activity in a novel environment was attenuated in the DBH-gene knockout (Dbh $^{-/-}$) mice (45). All prior results indicated a significant role of noradrenergic neurons in the exploratory behaviors of mice. The experiments performed in this study, with NE depletion after DSAP treatment, did not show disturbance of horizontal movements; however, consistent with prior reports, there were significantly fewer exploratory activities and of the rearing position as well. Discrepancies might be attributed to different treatment methods used to develop lesions of noradrenergic neurons. The data from the experiments performed in this study indicated that rearing behavior was significantly reduced; however, a wide range of horizontal activities (ambulation or stereotypes) showed no significant change. The underlying mechanism might be associated with ongoing posture changes from prostration to rearing in the neuropathic rats. As long as the orthostatic hypotension induced aversion (due to the orthostatic cardiovascular disturbances) without compensation, the rats would be reluctant to position themselves in upright postures.

Cerebral NE plays a significant role in sensory processing and behavioral performance (5, 45). The interaction between cardiovascular reactivity and posture changes, through cerebral noradrenergic mechanisms, might be relatively simple. In fact, previous electrophysiological studies in anesthetized rats demonstrated that an unpleasant stimulus could increase the firing, parallel and virtually identical activation of noradrenergic neurons in both the NE-LC and NE-SOA (22, 23). Similarly, previous studies reported marked persistent robust NE-LC that paralleled activation by loss of blood volume or hemorrhage. It is well known that such effects are essentially mediated by tonically active, vagal afferents from cardiac volume receptors (6, 11, 42).

Improved understanding of the importance of the central somatomotor-sympathetic system has brought into focus the relevance of cerebral noradrenergic mechanisms in cardiovascular regulation. It is unclear whether cerebral DBH deficiency is involved in orthostatic hypotension caused by changes in the modulation of RVLM, a final medullary relay for maintaining basal SOA and vasomotor homeostasis (12, 16, 28). Madden *et al*. (36) reported that after injection of DSAP directly into the RVLM, the sympatho-excitatory responses of rats appeared to be attenuated after a large and selective depletion of RVLM-adrenergic (C1) plus noradrenergic (A5) cell populations. However, the sympatho-inhibitory responses were apparently unaffected (36). This observation suggests that cardiovascular regulation could also be mediated by cerebral DBH in areas other than the RVLM. As already mentioned, an interaction between different suprabulbar brain regions and the RVLM is important for the overall effects on cardiovascular homeostasis (12, 16, 31, 32, 44). The results of the experiments in this study demonstrated a significant reduction in the resting BP and HR, in contrast to previous reports of unaffected HR. One possible explanation for the HR discrepancy could be the different experimental approaches. In the experiments performed in this study, a wide-ranging depletion of DBH and NE in various areas of the brain, including vagal motor neurons, might have augmented the activity of the parasympathetic outflow. Taken together, the findings suggest that cerebral noradrenergic neurons might play an integral

role in the somatomotor-sympathetic-cardiovascular system in maintaining the state-dependent vasomotor tonicity in response to stressful events such as orthostatic stress. In both humans and animals, cerebral DBH deficiency could be manifested as orthostatic hypotension, which is commonly observed in patients with peripheral DBH deficiency.

Effects of DBH Depletion on Behavioral Paradigms of Despair

Another finding of the present study was the association of NE depletion, after DSAP treatment, with moods, such as despair, in rats (20, 40). In an attempt to clarify mood-associated behaviors, despair and hedonic characteristics were assessed by the FST and SIT, respectively. The FST, as a test of mood, probes the role of various monoamine systems and receptor subtypes involved in stress-evoked depression (13, 14, 21, 39). Following initial periods of escape-oriented active behaviors, such as mobility, the animals showed behavioral immobility or passive floating, which is thought to reflect either the failure to persist in active behavior after persistent stress, or the development of passive behavior that releases the animal from coping with stressful stimuli. On the other hand, the lower levels of SIT or sucrose preference in stressed animals have been interpreted as a marker for anhedonia (43).

The data showed that after DSAP treatment, there was no difference in baseline mobility and immobility in the first one-week FST session; for the rest of the FST sessions, however, a significant reduction in the frequency of immobility with a corresponding increase in mobility activity reached the maximum at around 14 days and then vanished at around 21 days. On the other hand, DSAP treatment did not produce any abnormalities in sucrose intake, suggesting a stable pleasure response throughout the SIT session.

There were no striking FST changes observed in the experiments performed in this study; however, FST changes have been reported in other studies following the treatment of DSP-4 or gene knockout of DBH (13, 14). The reasons for the discrepancy remain unclear. Even with the different findings, however, all of the evidence implicates NE as an important factor in behavioral responses to inescapable stressful events, such as the FST used in this study. One mechanism could be associated with the dysregulated effects of degenerated noradrenergic neurons. Although the FST does not provide specific information on how degeneration of cerebral noradrenergic neurons alters other monoaminergic systems, the data from this study strongly favor intimate interaction among these systems (9, 17, 19, 27, 29, 30, 45). It is possible that the neurotoxic effects of DSAP are not restricted to noradrenergic terminal projections but also involve NE depletion at the cell body level, where changes in both the serotonin and dopamine systems occur. Such speculation is consistent with the view that NE has a significant influence on the serotonin system and that there is a reciprocal relationship between these two systems (24, 29, 30, 39). It is well known that there are reciprocal relationships between the dopamine and two other monoamine systems (9, 27, 45). It has been well-documented that abnormal dopamine mediates the pathogenesis of mood disorders, possibly in part by interactions with the serotonin and/or the NE system(s) (1, 19, 27). Further studies are needed to specifically determine the complex interactions involved with NE depletion.

To summarize, this study suggested that the involvement of central nervous system in cardiovascular homeostasis might be mediated by cerebral noradrenergic neurons. A widespread deficiency in cerebral DBH resulted in the overall depletion of epinephrine and NE in the brain, which might attenuate the sympatho-excitatory responses evoked by orthostatic stress. In addition, the results suggested that the depletion was relevant to mood disturbances, such as anhedonia or hopelessness. Clarifying the complex interactions of the central somatomotor-sympathetic system may be particularly important for understanding cardiovascular dysregulation and many degenerative disorders where cerebral DBH deficiency is present.

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