The flavonoids are usually present in the diet in relatively large amounts, which might be sufficient to achieve pharmacologically significant concentrations in tissues[2]. Thus, flavonoids could interfere with GSH S-transferase in vivo. Nevertheless, discrepances between in vitro and in vivo results have been described for some chemicals, such as propylthiouracil[25] or ellagic acid[26], which are in vitro inhibitors but in vivo activators of GSH S-transferase. Therefore the relevance of our results with respect to the influence of dietary flavonoids on GSH metabolism remains to be ascertained.

In conclusion, we have shown that flavonoids are potent in vitro inhibitors of GSH S-transferase and some structural features necessary for inhibition have been described. Further studies about the in vivo effect of several flavonoids on GSH metabolism are now in progress.

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Long-term depressor effects of catecholamine neuronal grafts in the third ventricle of the brain in normotensive rats

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Summary. Neuronal tissue containing A-6 group noradrenalin (NA) neurons of the locus ceruleus, or A-10 group dopamine (DA) neurons of the substantia nigra, was grafted into the third ventricle at the level of the preoptic-anterior hypothalamic region, in normotensive male rats. A significant and long-lasting depressor effect was shown in rats with either graft. In rats with an NA neuron-rich graft, plasma concentrations of arginine-vasopressin (AVP), plasma renin activity (PRA), and corticosterone (CS) decreased significantly, whereas in rats with a DA neuron-rich graft, AVP and PRA concentrations also decreased significantly but CS showed no significant change. Neither NA nor adrenalin in plasma changed significantly in rats with either graft.
Key words. Blood pressure; catecholamine; brain grafting; central cardiovascular regulation; rat.

With the help of anatomical and morphological studies to map the localization of catecholaminergic neurons and their receptors in the central nervous system (CNS)[1,2], physiology and pharmacology have accumulated evidence supporting the involvement of the CNS in cardiovascular regulation[3-11].

The anterior hypothalamic area (AHA) including the anteroventral third ventricle (AV3V) region[3-6,8], in cooperation with the nucleus of the solitary tract (NTS)[5,6], participates in body fluid and cardiovascular regulation mechanisms, mainly mediated by catecholaminergic neurons[5-11]. We have already reported that a long-term

depressor effect is induced by neural grafts containing NA neuron-rich tissue and DA neuron-rich tissue in the third ventricle at the AV3V level in rats made hypertensive by salt loading[12]. These depressor effects were thought to be due to an action of additional catecholaminergic input at the AV3V region.

The present study was designed to re-evaluate the depressor effect induced by these neural grafts in the third ventricle at the AV3V level in normotensive rats, and to identify the humoral agents that take part in the depressor mechanisms.

Materials and methods

Animals. Normotensive male Wistar rats 8 weeks old were used as recipients and pregnant rats of the same strain were used to obtain newborn rats as tissue donors. Animals were purchased from Japan Rat Laboratories, Urawa, Japan, and maintained under conditions of controlled temperature (24–26 °C) and illumination (lights on 05.00–19.00 h). Tap water and food pellets were provided ad libitum.

Grafting procedure. The tissue grafts, containing A-6 group noradrenalin (NA) neurons, from the locus ceruleus (NA neuron-rich tissue), or containing A-10 group dopamine (DA) neurons, from the substantia nigra (DA neuron-rich tissue), were punched out of coronal slice sections dissected from the medulla or midbrain of newborn rats, respectively, with a stainless metal cannula. The grafts were stereotaxically positioned in the third ventricle at the AV3V level in a recipient rat anesthetized with sodium pentobarbital. Tissue from the cerebellum was grafted as a control, and an empty cannula was inserted and pulled out for the sham control. All the procedures were done under sterile conditions.

Blood pressure (BP) measurement. To adapt the rats to the measuring conditions, BP was measured weekly for 4 weeks before the grafting experiment. The measurement began on the day of grafting and was repeated every 10 days. Systolic BP and heart rate (HR) were measured in conscious animals by an indirect tail cuff method using the rat-tail manometer system (KN-210, Natsume Seisakusho Co. Ltd., Tokyo, Japan). The animals were placed for 10 min on a warm plate to dilate the tail artery enough to measure BP. More than 3 readings were taken and averaged for each measurement.

Analytical methods. After BP readings were finished, the rats were decapitated and their brains were rapidly removed to be subjected to histological examination, as previously described[12,13]. Brain coronal sections were stained with luxol fast blue-cresyl violet. Only the data from the rats which were found to have a surviving graft in contact with the AV3V region were included in the results. It was confirmed in our previous study that such surviving grafts were always found to contain catecholamine (CA) neurons when the CA fluorescence method was used[13].

Immediately after decapitation of the rats, blood samples were collected on ice. They were then centrifuged, and the plasma was separated and frozen until the time of assay. Several humoral agents related to BP regulation, i.e., thyroid stimulating hormone (TSH), arginine-vasopressin (AVP), plasma renin activity (PRA), corticosterone (CS), NA and adrenalin (A) levels were measured as follows.

Serum TSH was measured by double-antibody RIA with a kit supplied by The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and results were expressed in terms of rat NIH-TSH-RP1. PRA was measured by solid phase RIA in which PRA can be measured in a single test tube throughout the enzymatic reaction and determination of angiotensin-I, which was generated with a kit obtained from Dainabot Radioisotope Laboratories Ltd. (Matsudo, Japan). AVP was measured by double-antibody RIA, using a reversed-phase C_{18} silica column for extraction, with a kit obtained from Mitsubishi Yuka Bio-Clinical Laboratories, Inc. (Tokyo, Japan). Plasma CS was determined by the protein-binding methods of Murphy[14] and Takahashi et al.[15] Plasma NA and A were measured by high performance liquid chromatography (HPLC) with electrochemical detection[16].

Statistical analysis. Two-way analysis of variance (ANOVA) and the new multiple range test of Duncan were applied to test the statistical significance of fluctuations in the mean BP and HR values. Student's t-test was applied to test the significance of the difference between the values in sham-operated control rats and in rats with NA or DA neuron-rich or cerebellum tissue grafts at corresponding days. Differences were considered statistically significant when $p < 0.05$ or 0.01.

Results

Histology. The present results were for the 16 out of 30 grafted animals in which survival and growth of the neonatal tissue was observed. The grafts demonstrated the morphological properties of normal tissue, and were well vascularized by vessels arising from the host hypothalamus. Normal glial and ependymal layers were absent.

Fluctuations in BP and HR. There were no significant BP changes in either sham-operated or cerebellum tissue-grafted controls, though slight fluctuations within 20 mm Hg occurred (fig. 1, upper panel). The rats with the grafts containing either NA neuron- or DA neuron-rich tissue showed a significant fall in BP, ranging between 20 and 45 mm Hg. This hypotension was present as early as 10–20 days after grafting and continued consistently during the 60-day period of BP measurement.

No significant HR change occurred in the sham-operated or cerebellum tissue-grafted control (fig. 1, lower panel). HR tended to decrease from 10 days after grafting both in rats with grafts of NA neuron-rich tissue and in those with DA neuron-rich tissue. Significant decreases of 25 to

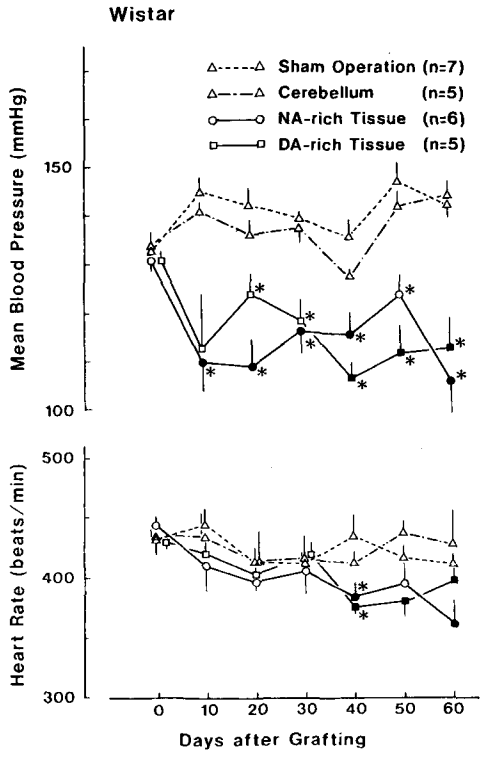

Figure 1. Graphs showing the effect of intracerebroventricular grafting of CA neuron-rich tissue derived from neonatal brain on the blood pressure (upper) and heart rate (lower) in rats. Tissues from the locus ceruleus and the substantia nigra are represented as NA-rich tissue and DA-rich tissue, respectively. Cerebellum tissue was grafted as the control, and an empty cannula was inserted and pulled out for the sham control. Values are shown as the mean + SEM for each tissue graft or control. The number of animals in each group is shown in parentheses. Solid point: $p < 0.05$ or 0.01 vs the control values (i.e. values on day 0) (applying the multiple range test of Duncan after ANOVA), *; $p < 0.05$ or 0.01 vs: the values in the animals with sham operations at corresponding times.

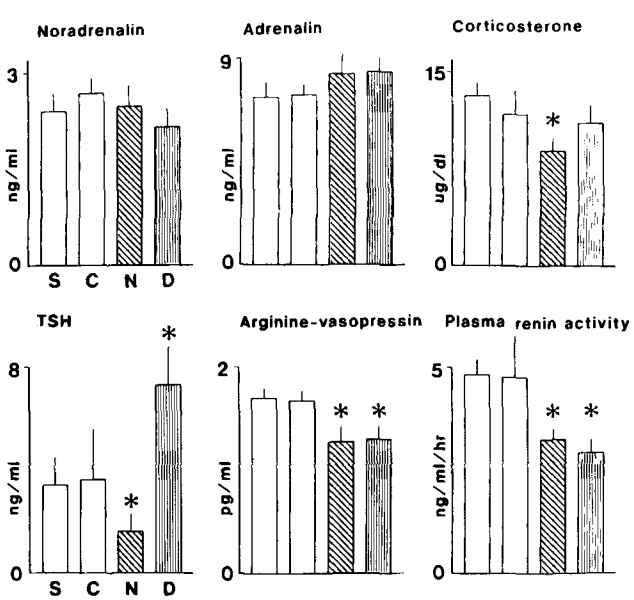

Figure 2. Graphs showing serum concentrations of noradrenaline, adrenaline, corticosterone, TSH, arginine-vasopressin, and plasma renin activity. Each bar represents the mean value and the vertical line indicates SEM. S: sham operated; C: cerebellum tissue grafted; N: NA-rich tissue grafted; D: DA-rich grafted group; *: $p < 0.05$ or 0.01 vs the values for the animal with sham operation.

50 bpm began at 40 days after grafting and continued until the end of the experiment. This bradycardia seemed to accompany the hypotension.

Characteristics of humoral agents. There were no significant differences between sham-operated and cerebellum tissue-grafted controls in the values for any of the humoral agents measured (fig. 2). Plasma AVP concentrations were significantly lower than those in control rats, both in rats with grafts of NA neuron-rich and in those with DA neuron-rich tissue. PRA values were also significantly lower than controls in both these groups. Plasma CS concentrations showed a significant decrease in rats with grafts of NA neuron-rich tissue, but there were no significant changes in those with DA neuron-rich tissue. No differences in either NA or A were seen among the groups, including controls. Significantly lower plasma concentrations of TSH were seen in rats with grafts of NA neuron-rich tissue, and higher TSH concentrations were found in rats with DA neuron-rich tissue.

Discussion

The present study demonstrated that either an NA neuron- or a DA neuron-rich graft in the third ventricle, in contact with the AV3V region, could produce a long-term decrease both in BP and in HR in normotensive rats, in association with decreases in humoral pressor agents such as AVP, PRA, and CS. The present data confirmed our previous observation in salt-loaded hypertensive rats[12], and further indicated the connection of such depressor effects of CA neuronal grafts with decreases in AVP, PRA and CS secretion.

Several reports have shown that both NA[7,9] and DA[10,11] administered into the AHA caused decreases in BP and HR, at doses such that they caused a reduction in sympathetic nerve activity[10]. Plasma CA concentrations may reflect sympathetic nerve activity as well as adrenomedullary activity[17], but in the present study neither NA nor A concentrations in plasma showed significant changes in any of the animal groups despite significant decreases in BP and HR. However, it was reported that intraventricular infusion of β-adrenergic blocking agents at small doses decreased BP and HR without parallel decreases in sympathetic nerve activity, suggesting that the drug-induced depression of sympathetic reflex outflow is counteracted when the carotid baroreceptors are intact[18]. Therefore, it seemed that both NA neuron- and DA neuron-rich tissue grafts were effective in decreasing BP and HR, but not in depressing the sympathetic reflex outflow.

The central action of NA has been to reduce corticotropin releasing hormone (CRH) and ACTH secretion[19-21], and subsequently to reduce CS secretion, probably through a central α-receptor[19,20]. Further, the adrenergic activity in the hypothalamus tonically depresses CRH activity[22]. In contrast, the functional role of central DA in the regulation of the CRH-ACTH-corticosteroid axis, if any, seems to be minimal[19,21]. The

present results agree with those reports, but it is not clear whether the decrease in plasma CS takes part in the mechanism of hypotension induction by NA neuron-rich tissue grafting.

NA has been shown to exert both stimulation[23,24] and suppression[25] of AVP release, possibly mediated by α-receptors[23-25]. Centrally administered DA also appeared to have either a stimulatory[25-27] or an inhibitory[25,28] effect on AVP release, probably through DA receptors[25-27]. These controversial results were probably due to species differences, effects of anesthesia, and differences in the subtype of the adrenoreceptor activated. The present results, showing that both NA neuron- and DA neuron-rich tissue grafts exerted an inhibitory influence on AVP secretion, suggested that CA transmission, both of NA and of DA, had an inhibitory effect on vasopressinergic magnocellular neurons.

In the regulation of plasma renin secretion, CA also plays important, probably multiple roles, through central CA receptors. The activation of α_1-receptor in the CNS stimulates renin secretion[29,30], but that of α_2-receptor inhibits it[29,30]. These effects are probably mediated by the neural pathway towards the kidney[30]. Centrally administered DA caused no change[31] in renin secretion, either stimulation[32] or inhibition[11]. The present results strongly suggested an inhibitory action of both NA and DA transmissions on renin secretion, although the precise mechanism is unknown. In addition, the present experiment, which demonstrated the decreases in both AVP and PRA at the same time, did not support the commonly accepted theory that AVP depresses renin secretion and may thus play an intermediary role in regulating PRA levels[33,34]. It is likely that the decrease in renin secretion is not mediated by AVP, but by other unknown mechanisms, which might include a direct effect of the CA neuron-rich tissue grafts.

The present study indicated that NA transmission at the level of the AHA region had an inhibitory effect on TSH secretion, but that DA transmission was facilitatory. These results seemed to be contrary to earlier reports that NA transmission participated in the stimulation of TSH secretion by stimulating the release of thyrotropin releasing hormone (TRH)[35,36], and that central DA inhibited TSH secretion[35,37]. However, these experiments were all performed with fairly high doses of CA, and thus it is probable that these controversial actions of CA were due to the dose of CA added. The reverse changes in TSH secretion in NA and in DA neuron-rich tissue grafted rats could indicate that both effects in the brain were independent of each other, so that it was unlikely that the effects obtained with DA neuron-rich tissue grafts were due to DA being converted to NA.

In summary, the present results suggest that catecholaminergic transmission in the forebrain is involved in cardiovascular regulation with hypotensive action, which could be, at least in part, mediated by inhibition of pressor agents, such as AVP and PRA.

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