diameter) lined with filter paper which was moistened with distilled water. All slugs were fed carrots and Iceberg lettuce, were maintained in a temperature controlled room (21 °C), and were exposed to natural lighting conditions.

All subjects were administered a single feeding preference test which consisted of presenting each slug with 2 square pieces of Iceberg lettuce (3 cm × 3 cm) during a 24-h period. Tests commenced at dusk. One piece of lettuce was dipped into distilled water (control group) and the other into a solution of blended slugs (experimental group). This solution was prepared in the following manner: 4 adult slugs were placed into a blender and mixed with 100 ml of distilled water until liquefied. The solution was then strained through 12-ply gauze sponges and immediately used for the feeding test.

For 3 consecutive days prior to the feeding test the slugs were fed a single 9-cm^2 piece of Iceberg lettuce. They were then food deprived for 36 h before the experiment. During the feeding preference test the 2 (control and experimental) squares of lettuce were placed 3 cm apart in the petri dish. The left-right position of the 2 lettuce squares were counterbalanced across subjects. Each pair of lettuce samples was cut from the same leaf and all squares were obtained from the same head of lettuce. Slugs were removed from the petri dishes during placement of the lettuce squares and when returned were positioned midway between the 2 samples.

After 24 h the remaining pieces of lettuce were removed and the percent consumed was calculated. This was determined by placing each remaining piece of lettuce under a grid marked off in 1 mm^2 and counting the number of 'squares' of lettuce consumed. Slugs were maintained in individual petri dishes on untreated lettuce for an additional 4 days and any mortality recorded.

Slugs consumed significantly ($p < 0.01$, 2-tailed Wilcoxon Test) more lettuce from leaves treated with distilled water than those treated with the blended slug solution. The mean ± SEM percentage consumed for the control lettuce was 44.0 ± 7.6, while only 6.1 ± 2.2% was eaten from the lettuce treated with blended conspecifics. One slug did not eat from either square of lettuce while the remaining 13 slugs all fed more from the lettuce treated with distilled water. Of these subjects, 38% fed only from the control lettuce and did not consume any of the blended slug treated lettuce. No mortality was observed during the 4 day post-experimental period.

This study has demonstrated that the terrestrial slug, *Limax flavus*, avoids food treated with a solution of blended conspecifics and water. This inhibition of feeding may be mediated through chemoreception[14,15] by detection of alarm substances. Since this study only examined the feeding responses of a single slug species to conspecific substances, it cannot be ascertained whether the phenomenon is species specific. The nature and source(s) of the substance(s) released, moreover, cannot be determined from this experiment because whole animals were blended. In a related species, *Lehmannia valentiana*, mucus from the dorsal surface of stressed slugs has been reported to be aversive to conspecifics[11]. The present experiment supports the contention of organic gardening enthusiasts who maintain that a solution of blended slugs and water will biologically control these garden pests[12,13].

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Effects of neurochemical lesions restricted to spinal cord monoaminergic neurons on blood pressure and sympathetic activity of spontaneously hypertensive rats

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Summary. Intraspinal (i.s.) injection of 6-hydroxydopamine or 5,7-dihydroxytryptamine in newborn spontaneously hypertensive rats (SHR) resulted, in the adult animal (30-week-old), in a marked decrease of spinal cord noradrenaline (NA) or 5-hydroxytryptamine (5-HT) levels, respectively. Since both neurotoxin- and vehicle-injected rats developed full hypertension and had similar plasma catecholamine concentrations, it is concluded that in SHR neither spinal cord NA nor 5-HT play a major role in development and maintenance of hypertension.

Studies performed in several animal models, including spontaneously hypertensive rats (SHR), indicate that both central noradrenaline (NA)- and 5-hydroxytryptamine (5-HT)-containing neurons are involved in the development of arterial hypertension[2-7]. Moreover, several experiments in which 6-hydroxydopamine (6-OHDA) and 5,6- or 5,7-dihydroxytryptamine (5,6-DHT, 5,7-DHT) have been injected i.c.v. support the view that both noradrenergic and serotoninergic neurons of the bulbospinal tract[9,10] participate in blood pressure regulation and development of hypertension[3,5,6,10,11]. However, neurotoxic lesions caused by i.c.v. injections of 6-OHDA, 5,6-DHT or 5,7-DHT, although relatively specific for NA- or 5-HT-containing neurons, are widespread in both brain and

spinal cord aminergic systems. In contrast, the intraspinal (i.s.) injection of 6-OHDA or 5,7-DHT in newborn rats elicit a rather selective, specific and long-lasting destruction either of NA or 5-HT neuronal terminals in spinal cord without damaging more rostral aminergic neurons projecting to the telediencephalon[12]. To evaluate more closely whether the noradrenergic and serotoninergic neurons projecting into the spinal cord play a role in the development of hypertension, in the following experiments 6-OHDA or 5,7-DHT was injected i.s. in newborn SHR in order to induce a degeneration of aminergic fibers in spinal cord terminals only.

Materials and methods. On the day of delivery, male and female pups of randomized litters of SHR were injected i.s. (8th thoracic intervertebral space) with either 6-OHDA · HCl (Fluka, 100 µg free base/rat), 5,7-DHT creatinine sulphate (Sigma, 25 µg free base/rat) or saline (controls, 1 µl vehicle/rat) as previously described[12]. All pups given 5,7-DHT had been injected i.p. 1 h previously with 20 mg/kg desmethylimipramine · HCl (DMI).

Adrenaline (A), NA, dopamine (DA) and 5-HT of 2 segments of the rat spinal cord (rostral and caudal to the injection site) were measured radioenzymatically[13,14] whereas 5-HT, NA and DA in the whole brain (including cerebellum and medulla oblongata) were determined fluorimetrically[12] 30 weeks after i.s. injection.

In some of the adult animals which had been cannulated previously into the common carotid artery[15], blood pressure (MAP) and heart rate (HR) were measured by Grass Polygraph recording. Resting plasma concentrations of NA and A, which are thought to represent a reliable biochemical index of sympatho-medullary activity[16], were measured radioenzymatically[13] in blood samples collected from the indwelling cannulas of 30-week-old SHR, freely moving and left undisturbed.

Statistical significance of differences between experimental groups was calculated by the Student t-test.

Results and discussion. In 30-week-old SHR injected with vehicle i.s. at birth (control animals) the amounts of 5-HT, NA and DA measured in the spinal cord (table) were quantitatively similar to those reported in the literature[17,18]. The highly sensitive radioenzymatic catechol-O-methyltransferase assay used in our study permitted also a reliable measurement of A. As shown in the table, this amine is present, although in minute amounts (1.3 ng/g tissue) in the spinal cord, supporting the view that, in the rat, only few A-containing neurons project to the spinal cord[19].

In the thoraco-lumbar segment of the cord, the relative percentage contribution of NA, DA and A to the total catecholamine content correspond to 94, 5.5 and 0.5%, respectively. In 30-week-old SHR injected i.s. at birth with 6-OHDA, the catecholamine content was decreased more markedly in the thoraco-lumbar than in the thoracic segment of the cord (table). Apparently, the neurotoxin induced a specific and long-lasting denervation of catecholamine pathways exclusively in the spinal cord. Indeed, following i.s. 6-OHDA 5-HT in the spinal cord as well as NA, DA and 5-HT content of the brain remained unaffected (table). The observation that DA and A levels were reduced less markedly than those of NA following 6-OHDA, support the view that these 3 catecholamines are stored in different neuronal systems with specific physiological functions[19]. As previously shown by other authors[20, 21] our results support the concept that spinal cord DA and A neurons are more resistant to the neurotoxic effects of 6-OHDA than NA-containing neurons. After neonatal i.s. injection of 5,7-DHT, a marked decrease of 5-HT, but no change in the catecholamine content, were found in the spinal cord of 30-week-old SHR.

The 5,7-DHT-induced denervation resulted in a long-lasting and specific decrement of 5-HT only in the spinal cord. Indeed, by this treatment spinal cord catecholamines as well as brain 5-HT, NA and DA levels were virtually unaffected (table).

Brain and spinal cord monoamine levels, plasma catecholamine concentrations, mean arterial pressure (MAP) and heart rate (HR) of 30-week-old spontaneously hypertensive rats (SHR) injected at birth with 6-hydroxydopamine (6-OHDA) or desmethylimipramine (DMI) plus 5,7-dihydroxytryptamine (5,7-DHT)

	Vehicle-injected controls	6-OHDA	5,7-DHT
Brain 5-HT (ng/g)	539 ± 17	579 ± 50	546 ± 7
Brain NA (ng/g)	447 ± 37	416 ± 18	454 ± 19
Brain DA (ng/g)	723 ± 43	771 ± 48	766 ± 25
Spinal cord A (ng/g) T_3–T_8	1.3 ± 0.1	0.8 ± 0.1	1.1 ± 0.2
T_8–L_2	1.3 ± 0.3	0.3 ± 0.03*	0.8 ± 0.1
Spinal cord NA (ng/g) T_3–T_8	212 ± 24	5.7 ± 1.2**	242 ± 44
T_8–L_2	246 ± 17	1.8 ± 0.5**	232 ± 31
Spinal cord DA (ng/g) T_3–T_8	18 ± 1.8	12 ± 2.8	21 ± 2.5
T_8–L_2	15 ± 2.7	2.6 ± 1.2**	9.8 ± 0.8
Spinal cord 5-HT(ng/g) T_3–T_8	437 ± 49	349 ± 64	32 ± 7**
T_8–L_2	877 ± 101	700 ± 85	23 ± 8**
Plasma A (pg/ml)	80 ± 15	74 ± 16	65 ± 10
Plasma NA (pg/ml)	310 ± 30	369 ± 118	369 ± 118
MAP (mm Hg)	198 ± 5	190 ± 4	200 ± 5
HR (beats/min)	490 ± 13	444 ± 12	460 ± 11

Pups were injected free-hand with 1 µl of the vehicle (saline containing 0.1% ascorbic acid) without or with 6-OHDA (100 µg free base) or with 5,7-DHT (25 µg free base 1 h after 20 mg/kg DMI i.p.) into the spinal cord at the level of the 8th thoracic intervertebral space[12]. T, thoracic; L, lumbar. Figures represent means ± SEM of 5 single values (1 rat each). Statistical significance for difference from corresponding control (Student t-test): *$p < 0.05$; **$p < 0.01$.

On the whole, the present biochemical results clearly show that neonatal i.s. injection of 6-OHDA or 5,7-DHT provide an animal model characterized by a marked impairment of intraspinal catecholaminergic or serotoninergic pathways, respectively.

In 30-week-old SHR injected i.s. at birth with saline, 6-OHDA or 5,7-DHT, both MAP and HR as well as plasma A and NA concentrations were measured. As shown in the table (lower part), no significant differences in MAP or HR-values were observed among the animals injected neonatally either with vehicle, or with 6-OHDA or 5,7-DHT.

All SHR developed full hypertension and also had similar plasma concentrations of A and NA (table I) supporting the view that, in spite of the different neurotoxic lesion, the sympatho-medullary activity did not differ from that of untreated adult SHR[22].

It is generally accepted that the depletion of spinal cord NA in 6-week-old animals by 6-OHDA does not delay the onset and the development of hypertension of SHR[5,23].

Moreover, the present study shows that a marked reduction of either NA or 5-HT in the spinal cord of newborn SHR does not affect the hypertensive state or the long-term modulation of the sympatho-medullary activity. On the whole, it can be concluded that the development and the maintenance of hypertension in SHR does not require the integrity of the spinal cord noradrenergic or serotoninergic systems.

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Neurotransmitter receptors as glycoproteins

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Summary. Incubation of calf brain membrane preparations with the plant lectins, concanavalin A and wheat germ agglutinin did not inhibit neurotransmitter receptor binding sites directly. Plant lectins did however protect these sites against subsequent trypsin digestion suggesting that neurotransmitter binding sites may be associated with glycoprotein structures.

The structure of the neuronal cell surface plasma membrane determines not only the response of the neurone to transmitters and hormones but also their interaction with exogenous agents such as drugs, toxins, antibodies and viruses. In recent years glycoproteins have been shown to have a central role in interactions with such exogenous agents[1]. Glycoproteins are present in significant amounts on the cell surface[2,3], and may be concentrated in synaptic regions[4,5]. In brain it has been estimated that 80–90% of cell glycoproteins are membrane bound[4], but little is known of the specific functions of these membrane components. Several hormone receptors have been identified as glycoproteins[6], and the nicotinic acetylcholine receptor complex includes a number of glycoprotein subunits[7]. The present study was undertaken to determine if other neurotransmitter receptors include carbohydrate residues. A general rule that receptors are glycoproteins would suggest a wider involvement of transmitter receptors in cell recognition processes. Many of the properties of membrane bound glycoproteins have been elucidated by the use of plant lectins, the interaction with lectins involving the recognition of specific carbohydrate sequences[9]. We have therefore examined the direct and indirect interactions of plant lectins with a number of ligand binding sites in calf brain.

Materials and methods. Calf cerebral membranes were prepared as described previously[10], tissues were homogenized in 40 vol. of 50 mM tris/HCl buffer pH 7.4 and centrifuged at 50,000 × g for 20 min. The pellets were washed once and resuspended in the original volume of buffer to give a crude membrane preparation. Membranes were incubated in the presence or absence of plant lectin (see fig. 3) at 37 °C for 30 min. Trypsin was added at the appropriate concentration and the incubation continued for 15 min. Soyabean trypsin inhibitor was added and aliquots taken for the determination of ligand binding. Binding assays were performed using established techniques adapted for semi-automated analyses[11] using the following conditions: ^{3}H-N methyl scopolamine binding to muscarinic receptors[12] (^{3}H-NMS, ligand concentration 0.5 nm displaced by 10 μM atropine). ^{3}H-spiperone binding to dopamine D2 receptors[13] (^{3}H-SPIP, 0.5 nm displaced by 1 μM (+) butaclamol), ^{3}H-dihydroalprenolol binding to β-adrenoceptors[14] (^{3}H-DHA, 2.5 nM displaced by 1 μM propanolol), ^{3}H-WB4101 binding to α-adrenoceptors[15] (^{3}H-WB4101, 2 nM displaced by 1 μM aceperone), ^{3}H-pyrilamine binding to histamine H1 receptors[16] (^{3}H-PYR, 5 nm, displaced by 1 μM pyrilamine), ^{3}H-flunitrazepam binding to benzodiazepine receptors[17] (^{3}H-FNZ 1.5 nM displaced by 1 μM clonazepam). ^{3}H-etorphine binding to opiate receptors[18] (^{3}H-ETOR 2 nM displaced by 1 μM naloxone), and ^{3}H-muscimol binding to GABA receptors[19] (^{3}H-MUSC, 7 nM displaced by 10 μM GABA). All assays were performed with calf cortical membranes except ^{3}H-SPIP which used calf caudate membranes.

Results and discussion. Preliminary studies demonstrated that incubation of calf brain membrane preparations with the lectins given in figure 3 had no effect on the subsequent binding of any of the ligands used. When calf cerebral cortex membrane preparations were preincubated with trypsin, a rapid and almost complete loss of ^{3}H-N methyl scopolamine (^{3}H-NMS) binding to muscarinic cholinergic receptors was observed. In subsequent experiments a concentration of trypsin producing approximately 80% loss of ^{3}H-NMS binding was used (fig. 1). Incubation of membranes with the lectin concanavalin A (Con A), prior to trypsin treatment resulted in a significant protection of ^{3}H-NMS binding (fig. 1). When α-methyl mannoside (a hapten for Con A) was included in the Con A preincubation, the protective effect of Con A was no longer observed. α-Methylmannoside alone had no effect on the binding of ^{3}H-NMS, or on the reduction of ^{3}H-NMS binding by trypsin. Thus the action of Con A depends upon its affinity for a specific carbohydrate structure. Treatment of membranes with neuraminidase (an enzyme which removes