Dopamine β-Hydroxylase and Tyrosine Hydroxylase Activities in Spontaneously Hypertensive Rats after NaCl Administration

It was found[1,2] that serum dopamine β-hydroxylase (DBH) activity of spontaneously hypertensive (SH) rats[3] is elevated only when they are very young, then decreases during the development of hypertension to a level similar to that of normotensive Wistar-Kyoto rats, but increases again significantly when the blood pressure is increased rapidly by NaCl administration.

In order to investigate the origin of elevated dopamine β-hydroxylase in serum of SH rats after NaCl administration, the DBH activity in sympathetically innervated tissues, such as mesenteric vessels and vas deferens, as well as in adrenal glands, has been examined with SH rats after NaCl administration and compared with that of control SH rats without NaCl administration. Tyrosine hydroxylase (TH) activity, which is known to be increased when DBH activity is increased[4], has also been measured in the mesenteric vessels, vas deferens, adrenal glands and brain stem.

DBH and TH activities in the tissues, from the same SH rats as reported in our previous paper[2], have been examined. NaCl was administered to SH rats by supplying 1% NaCl-aqueous solution for drinking instead of tap water after 10 weeks of age for 4 weeks[2]. Rats were decapitated, and mesenteric vessels, vas deferens, adrenal glands, and brain were quickly removed, weighed, frozen on dry ice, and stored at −80 °C. 'Mesenteric vessels' consist of superior, inferior and coeliac mesenteric arteries and veins plus connective tissues after removing fat from the mesentery. DBH and TH were isolated by ammonium sulfate fractionation as described before[4,5]. The frozen tissues were homogenized in 2–4 ml of 0.1 *M* phosphate buffer (pH 7.5). The homogenate was centrifuged at 100,000 × *g* for 30 min. TH was isolated from the supernatant by fractionating it with ammonium sulfate (0–40% fraction). DBH was solubilized from the particulate fraction with a detergent Cutscum and separated with ammonium sulfate (80% saturation). TH activity was determined by the formation of ^{14}C-dihydroxyphenylalanine from L-^{14}C-tyrosine[6]. The incubation mixture (total volume 1.0 ml) contained: 200 μmol of acetate buffer, pH 6.0, 0.1 μmol of L-tyrosine containing 8.0×10^4 dpm of L-[U-^{14}C]tyrosine, 100 μmol of mercaptoethanol, 1.0 μmol of 6,7-dimethyltetrahydropterin, 1.0 μmol of $FeSO_4$, and an appropriate amount of the enzyme preparation. Incubation was carried out at 30 °C for 15 min. Nonincubated zero-time blank was taken as control. DBH activity was determined by sensitive dual-wavelength spectrophotometry using tyramine as substrate[7]. The incubation mixture (total volume 1.0 ml) contained: 400 μl of enzyme, 200 μmol of sodium acetate, pH 5.0, 10 μmol of N-ethylmaleimide, 1 nmol of $CuSO_4$, 10 μmol of sodium fumarate, 10 μmol of ascorbic acid, 1 μmol of pargyline, 1500 units (50 μg) of catalase, and 20 μmol of tyramine. Incubation was carried out at 37 °C for 45 min. A sample of boiled (95 °C, 5 min) enzyme preparation was used as blank.

As reported in our previous paper[2], the blood pressure of control SH rats without NaCl administration increased from 170 ± 3 mm Hg (at 10 weeks) to 189 ± 3 mm Hg (at 14 weeks), whereas that of SH rats with NaCl administration was increased from 173 ± 2 mm Hg (at 10 weeks) to 204 ± 5 mm Hg (at 14 weeks). Increase in blood pressure in SH rats with NaCl administration was significantly higher than that in control SH rats without NaCl. The blood pressure of Wistar-Kyoto rats at 14 weeks was 123 ± 7 mm Hg.

DBH and TH activities in tissues of normotensive Wistar-Kyoto rats, from which strain SHR were bred[2], at 14 weeks and those of SH rats at 14 weeks without and with NaCl administration are shown in the Table.

Mean DBH and TH activities in adrenals of SH rats without NaCl were about 2-fold higher than those of Wistar-Kyoto rats. This agrees with our previous results showing increased DBH and TH activities in adrenals of SH rats as compared with those of normotensive Wistar rats[4,5]. The increase in adrenal TH activities of SH rats compared with those of Wistar-Kyoto rats was statistically significant. However, because of variation in adrenal DBH activities, the increase in adrenal DBH activities in

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Dopamine β-hydroxylase and tyrosine hydroxylase activities of spontaneously hypertensive (SH) rats

Enzyme activity (nmol/min/g ± SEM)	Wistar-Kyoto rats	SH rats without NaCl	SH rats with NaCl
Dopamine β-hydroxylase			
Serum[a]	0.19 ± 0.04 (6)	0.19 ± 0.03 (6)	0.30 ± 0.07 (10)[c]
Adrenals	12.4 ± 3.3 (3)	21.3 ± 8.6 (6)	28.0 ± 6.2 (10)
Mesenteric vessels	0.12 ± 0.01 (3)	0.24 ± 0.07 (6)	0.66 ± 0.15 (4)[c]
Vas deferens	1.26 ± 0.45 (3)	1.39 ± 0.25 (6)	1.53 ± 0.16 (10)
Tyrosine hydroxylase			
Adrenals	7.95 ± 0.80 (3)	15.3 ± 2.6 (6)[b]	16.0 ± 1.8 (10)
Mesenteric vessels	0.14 ± 0.02 (3)	0.19 ± 0.01 (6)	0.40 ± 0.05 (8)[c]
Vas deferens	2.94 ± 0.02 (3)	3.92 ± 0.29 (6)[b]	3.70 ± 0.25 (10)
Brain stem	0.75 ± 0.06 (3)	1.08 ± 0.09 (3)	0.89 ± 0.03 (3)

[a] Reported in the previous paper[2]. [b] Differs from control (Wistar-Kyoto rats), $p < 0.01$. [c] NaCl was administered from 10 weeks of age for 4 weeks. Differs from control (SH rats without NaCl administration), $p < 0.05$.

SHR was not statistically significant. TH activity in vas deferens of SH rats was also higher than that of Wistar-Kyoto rats. DBH activities in mesenteric vessels and vas deferens and TH activities in mesenteric vessels and brain were not statistically different between Wistar-Kyoto rats and SH rats without NaCl administration.

When NaCl was administered to SH rats, DBH and TH activities in mesenteric vessels were increased significantly about 2–3 fold. In contrast, the increase in both enzyme activities in adrenals and vas deferens was not statistically significant. These results suggest that the elevated serum DBH activity after NaCl may be mainly derived from the blood vessels, and are consistent with the concept that sympathetic nerve activity may be increased in blood vessels during the rapid increase of blood pressure by NaCl administration in SH rats[8].

Zusammenfassung. Nachweis, dass Dopamin-β-Hydroxylase-Aktivität und Tyrosin-Hydroxylase-Aktivität in Mesenterialgefässen spontan hypertoner Ratten nach Kochsalzgaben im Trinkwasser erhöht sind.

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Gewebekulturen aus Alkaloidpflanzen IV.[1] *Macleaya microcarpa* (Maxim.) Fedde

Tissue Cultures of Alkaloid Plants IV.[1] *Macleaya microcarpa* (Maxim.) Fedde

Die Papaveracee *Macleaya microcarpa* enthält wie die nahe verwandte *M. cordata* die Hauptalkaloide Protopin und Allokryptopin sowie das gefärbte Sanguinarin[2]. Diese Alkaloide werden auch in isolierten Kalli von *M. cordata*-Explantaten gebildet und sind dort in einzelnen, gelb gefärbten Zellen angereichert[3,4]. Eine Überführung des Kallusgewebes in Dauerkultur erfolgte bisher nicht. Es ist aber wesentlich zu prüfen, ob die Fähigkeit zur Alkaloidbildung bei fortlaufender Subkultivierung erhalten bleibt. Wir berichten daher über Untersuchungen an mehrjährigen Gewebekulturen von *M. microcarpa*.

Material und Methoden. Stengel (⌀ 3–8 mm) von *M. microcarpa*-Pflanzen im Knospenstadium wurden nach Sterilisieren mit gesättigter Chlorkalklösung (30 min) in 1,0–1,5 cm lange Stücke geschnitten und auf den mit Agar (8 g/l) verfestigten Nährmedien 1a, 1b und 2 (Tabelle) bei 25 °C und 12 h/Tag Fluoreszenzlicht (1000–1200 Lux) gehalten. Nach 9 Wochen wurden die gebildeten Kalli vom Explantat getrennt und selbständig kultiviert. In Abständen von 3 Wochen erfolgte eine Übertragung auf neues Nährmedium.

Die Alkaloidextrakte aus Gewebe- und Pflanzenmaterial wurden in üblicher Weise gewonnen und gereinigt. Zur Erfassung ausgeschiedener Alkaloide wurden die Nährmedien homogenisiert, alkalisiert und erschöpfend mit Chloroform extrahiert. DC der gereinigten Extrakte erfolgte an Kieselgel G (Merck)-Schichten mit Benzol/Methanol (8:2). Zur kombinierten quantitativen Allokryptopin/Protopin-Bestimmung wurden die entsprechenden, unbehandelten DC-Zonen isoliert. Lage und Grösse der Allokryptopin- und Protopin-Flecke wurden durch Betrachten unter UV-Licht und mit Hilfe angefärbter Vergleichssubstanzen auf demselben DC ermittelt. Die alkaloidhaltigen Kieselgelproben wurden durch Zugabe von Kieselgel G auf ein Gewicht von 500 mg gebracht und mit 0,3 ml Essigsäure (99%ig) versetzt. Nach Auffüllen mit konzentrierter Schwefelsäure zu einem Gesamtvolumen von jeweils 10 ml wurden die Proben 60 min bei 80 °C im Wasserbad gehalten und bei 534 nm gegen eine Nullprobe mit 500 mg alkaloidfreiem Kieselgel G vermessen. Von jedem Material wurden Proben mit unterschiedlichen Alkaloidmengen zur Messung gebracht. Da das Verhältnis von Protopin zu Allokryptopin nicht genau bekannt war, wurden nur Messungen, deren Extinktionen im Schnittbereich der Eichkurven beider Alkaloide lagen, ausgewert.

Die lichtmikroskopischen Untersuchungen erfolgten an Handschnitten von Gewebekulturen, die in erkaltende Agarlösungen (2–5%ig) bei 48 °C eingebettet wurden. Als Fällungsreagenz für Alkaloide diente Jod-Jodkalium (Mandel-Reagenz, 10%ig).

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Zusammensetzung der verwendeten Nährmedien

Mineralstoffe	Vitamine und Aminosäuren nach KOBLITZ und HAGEN[7]	Vitamin B_1 (ppm)	2.4-D (ppm)	IES (ppm)	Kinetin (ppm)	Gibberellinsäure (ppm)	Äpfelsäure (ppm)
1a. MURASHIGE und SKOOG[5]	+	0,3	1,0	—	0,2	0,1	50
1b. MURASHIGE und SKOOG[5]	+	0,3	—	10,0	0,4	0,1	50
2. HELLER[6]	+	0,3	—	2,0	0,2	0,1	50