ditation[16] or carcinoid flush[17], respectively, may be answered if the activity of kinins on blood vessels depends on release of a mediator(s) having similar or even antagonistic actions to kinins[18].

Zusammenfassung. Nachweis, dass Bradykinin den renalen Blutstrom um 58% der Kontrollwerte (282 ± 40 ml/min) erhöht und gleichzeitig im venösen Nierenblut die Konzentration einer Substanz, welche die physicochemischen und biologischen Eigenschaften eines Prostaglandins der E-Serie besitzt, steigert, wobei die Konzentration einer PGF-ähnlichen Substanz unverändert blieb.

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[16] K. D. Bhoola, J. Morley, M. Schachter and L. H. Smaje, J. Physiol., Lond. 179, 172 (1965).

[17] J. A. Oates, W. A. Pettinger and R. B. Doctor, J. clin. Invest. *45*, 173 (1966).

[18] This work was supported by grants from the U.S. Public Health Service and the Missouri and American Heart Associations. We thank the Upjohn Co. for its generosity in supporting this investigation.

Effect of 5-Hydroxydopamine on Uptake and Content of Serotonin in Rat Striatum

The synthetic phenylethylamine derivative 5-OH-dopamine is able to act as a false transmitter, i.e. it can replace norepinephrine in the storage vesicles[1,2] and is released from sympathetic nerves[1]. The advantage of 5-OH-dopamine in animal experiments is its intense osmiophilic property, so that the replacement of norepinephrine can be observed with the help of the electron microscope. Like L-dopa, the analogue 5-OH-dopa is also decarboxylated to the respective amine, i.e. 5-OH-dopamine. It is known that treatment of several species with L-dopa causes a decrease in the serotonin (5-HT) content of brain[3–6], which might be due to enhanced release[3,7] and metabolism[3] of 5-HT. Therefore, it was of interest to know whether 5-OH-dopa acted similarly to L-dopa. The serotonin content of the striatum was measured in rats after treatment with 5-OH-dopa. Further, the influence of 5-OH-dopamine on the uptake of tritiated serotonin by striatal tissue slices of rats was studied in vitro.

Materials and methods. 1. In the in vivo experiments (male Sprague-Dawley rats, 120–150 g) 5-OH-dopa was injected twice in a dose of 400 mg/kg i.p. 15 and 4 h before decapitation. The serotonin content of rat striatum was determined according to the method of Snyder et al.[8].

2. In vitro, striatal tissue slices of rats, prepared according to McIlwain et al.[9], were incubated at 37 °C in a glucose Ringer's solution with addition of 0.2 mg/ml ascorbic acid and under constant gas supply (95% O_2, 5% CO_2). After 10 min of pre-incubation ^{3}H-serotonin (spec. activity 8.5 C/mM, Radiochemical Center, Amersham) was added to the incubation medium to obtain a final concentration of $5.9 \times 10^{-7} M$. After 30 min of incubation the slices were rinsed in fresh buffer and blotted with filter paper. The slices were weighed and solubilized in 0.5 ml Soluene TM_{100} (Packard Instr. Co. Inc.). Radioactivity of each tissue slice was counted in a liquid scintillation spectrometer (LS-200B, Beckman). The uptake of ^{3}H-5-HT was determined by calculating the tissue to medium ratio of the isotope after correction by the external standard ratio method. 5-OH-dopamine was added after the preincubation period in concentrations of 0.001–1.0 mg/ml.

3. For electron microscope preparation, the incubated tissue slices (see above) were rapidly fixed in glutaraldelhyde fixative with phosphate buffer (pH 7.4) for 1 h. They were then washed in phosphate buffer and post-fixed in

[1] H. Thoenen, *Experimentelle Medizin, Pathologie und Klinik* (Springer-Verlag, Berlin 1969), vol. 27, p. 32.

[2] J. G. Richards and J. P. Tranzer, Experientia *25*, 53 (1969).

[3] G. M. Everett and J. W. Borcherding, Science *168*, 849 (1970).

[4] G. Bartholini, A. Pletscher and W. P. Burkhard, J. Pharm. Pharmac. *20*, 228 (1968).

[5] A. Bertler and E. Rosengren, Experientia *15*, 382 (1959).

[6] M. Karobath, J.-L. Diaz and M. O. Huttunen, Europ. J. Pharmac. *14*, 339 (1971).

[7] K. Y. NG, T. N. Chase, R. W. Colburn and I. J. Kopin, Science *170*, 76 (1970).

[8] S. H. Snyder, J. Axelrod and M. Zweig, Biochem. Pharmac. *14*, 831 (1965).

[9] H. McIlwain and R. Rodnight, *Practical Neurochemistry* (Churchill, London 1962), p. 109.

Uptake of ^{3}H-serotonin ($5.9 \times 10^{-7} M$) by striatal tissue slices of rats in vitro under the influence of 5-OH-dopamine

	Control	5-OH-dopamine (μg/ml)			
		1000	500	10	1
Uptake ratio $\bar{x}$ ± S.E.M.	11.85 ± 0.87	3.75 ± 0.15	4.06 ± 0.26	6.86 ± 0.47	9.37 ± 0.37
No. of experiments	15	10	13	12	8
% of control	100	32	34	58	79
P		< 0.001	< 0.001	< 0.001	< 0.02

For details, see methods. p was calculated according to the Student's t-test.

1% osmium tetroxide fixative for another hour, dehydrated in graded ethanol and propyleneoxide, embedded in Epone 812 and polymerized.

The tissue blocks were sectioned with LKB ultramicrotome, contrasted in lead nitrate and uranyl acetate and observed under Siemens Electronmicroscope 1A.

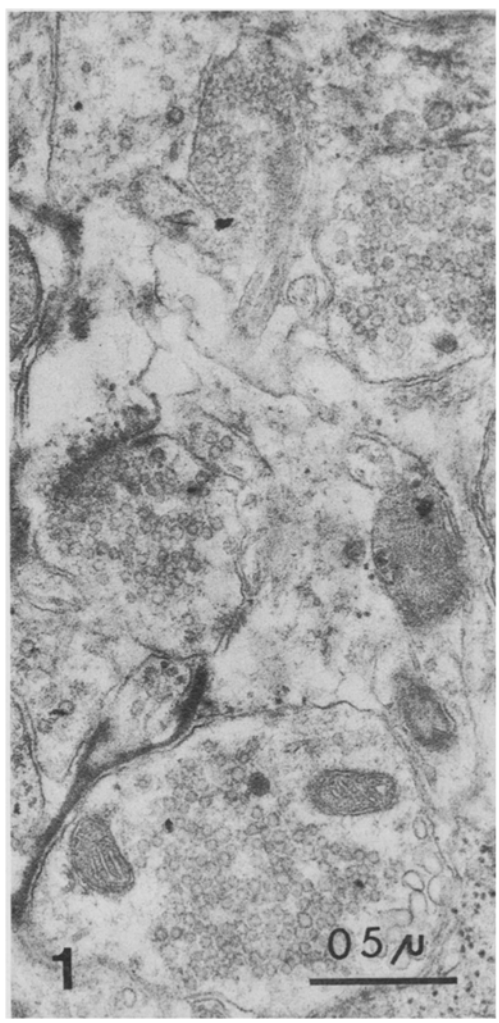
Results. In vivo, treatment of the animals with 5-OH-dopa lowered the content of serotonin in the striatum of rat brains significantly from 0.51 ± 0.03 to 0.39 ± 0.02 µg/g wet wt. ($n = 5$, $p < 0.01$), i.e. by about 25 %.

Addition of 5-OH-dopamine to the incubation medium significantly reduced the uptake of tritiated serotonin by striatal tissue slices of rats, when compared with the control uptake (Table). The inhibition of serotonin uptake by 5-OH-dopamine was dose-dependent with a maximum inhibition of about 70% in a concentration of 1 mg/ml of 5-OH-dopamine; 1 µg/ml inhibited serotonin uptake by about 20%. Also dopamine dose-dependently decreased serotonin uptake in concentrations of 1.5–150 µg/ml (unpublished results).

When striatal tissue slices were incubated with 1 mg/ml 5-OH-dopamine under the conditions described above we were unable to demonstrate a morphological change in any nerve terminals (Figure 1). However, using labelled dopamine, a fine accumulation of silver grains could be observed in these slices by autoradiographic studies. Nevertheless, a clear cut substructural change by accumulation of electron dense material in dense core vesicles could be demonstrated in peripheral sympathetic nerve terminals of pineal glands of rats incubated with the amine (Figure 2). This is in accordance with results in vivo experiments[2].

Discussion. The results presented show that 5-OH-dopamine and dopamine are able to decrease the uptake of tritiated serotonin in striatal tissue slices of rats in vitro. Like dopa, 5-OH-dopa also lowers the serotonin content of rat striatum when injected in vivo. A decrease in serotonin content of the brain after 5-OH-dopa was described, as far as we know, only in *Lacerta viridis*[10]. Thus, there seems to be a striking analogy between the serotonin lowering effect of L-dopa[3–7] and that of the synthetic analogue 5-OH-dopa. Histochemical observations suggest that dopamine, which is formed from exogenous L-dopa, can accumulate in dopaminergic as well as in serotoninergic

[10] H. G. BAUMGARTEN, H. BRAAK and H. WARTENBERG, Z. Zellforsch. *95*, 396 (1969).



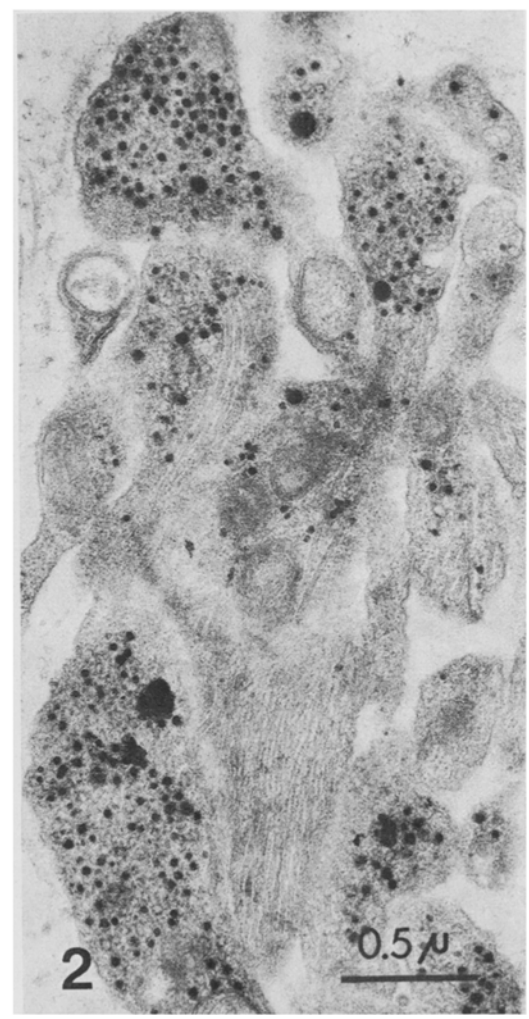


Fig. 1. Electron micrograph taken from striatal tissue slice incubated in 1 mg/ml 5-OH-dopamine containing medium for 30 min, demonstrates no substructural changes in the nerve terminals.

Fig. 2. Electron micrograph taken from pineal gland, incubated in 1 mg/ml 5-OH-dopamine containing medium for 30 min, demonstrates accumulation of electron-dense material in dense core vesicles (500 Å) and in large granulated vesicles (1000 Å) in sympathetic nerve terminals.

neurons[11]. Decarboxylation of exogenous L-dopa to dopamine seems to be necessary for resultant displacement of the endogenous serotonin from the vesicular stores[7]. On the other hand, experimental data give evidence that the decrease in serotonin content of rat brain after administration of L-dopa may be caused by reduction of the concentration of its precursor, tryptophan[6]. These possibilities of interactions may also be true in the case of 5-OH-dopa as the compound is metabilized to 5-OH-dopamine[1].

Further interactions between the catecholamines, 5-OH-dopamine and dopamine, and the indoleamine, serotonin are also demonstrated by the uptake studies described here. Both catecholamines dose-dependently decrease the amount of tritiated serotonin accumulated by striatal tissue slices in vitro. It is assumed that the decrease in the uptake of 5-HT is not due to the inhibition of the uptake mechanism for 5-HT, but that displacement of serotonin by the catecholamines takes place at the storage sites. This assumption is supported by kinetic studies with striatal tissue slices of rats in vitro. These investigations indicate the presence of at least 2 distinct uptake mechanisms for serotonin, thereby showing that serotonin may also enter catecholamine containing neurons, depending on the concentrations of 5-HT present[12]. This non-specific uptake of 5-HT into catecholaminergic terminals is also indicated by experimental studies with isolated P_2-fractions from hypothalamus and corpus striatum of rats[13]. To what extent the decrease in serotonin accumulation by 5-OH-dopamine and dopamine is due to displacement of non-specifically accumulated serotonin from the catecholaminergic terminals, and/or due to discplacement of serotonin from serotoninergic terminals, needs further elucidation. Nevertheless, this problem seems mainly to be a question of concentrations both of the catecholamines and of the indoleamine.

Zusammenfassung. 5-OH-Dopa und 5-OH-Dopamin senken in vivo den Serotoningehalt und vermindern in vitro dosisabhängig die Aufnahme von ^{3}H-Serotonin im Striatum von Ratten. Es kann daher angenommen werden, dass 5-OH-Dopamin in vivo überwiegend eine Verdrängung von Serotonin aus den serotoninergen Speichern verursacht, während in vitro zusätzlich eine Verdrängung von unspezifisch in katecholaminerge Speicher aufgenommenem Serotonin auftreten kann.

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[11] L. L. Butcher, J. Engel and K. Fuxe, J. Pharm. Pharmac. *22*, 313 (1970).
[12] E. G. Shaskan and S. H. Snyder, J. Pharmac. exp. Ther. *175*, 404 (1970).
[13] K. Takatsuka, T. Segawa and H. Takagi, J. Neurochem. *17*, 695 (1970).

Tissue Culture of *Nigella sativa* I. The Behaviour of Nucleus

The stability of chromosome number throughout a culture regime of $3^1/_2$ years in callus tissue of *Nigella sativa* is reported here.

Materials and methods. Callus tissue derived from the stem of *Nigella sativa* ($2n = 12$) was grown in modified tobacco medium[1] supplemented with NAA (0.05 mg/l) and coconut milk 15% (v/v). The medium was solidified with 0.7% bacto agar. Friable strain with faster growth was selected and transferred to the liquid medium. Tissues were grown in 250 ml. Erlenmeyer flask containing 25 ml medium on a gyro-rotatory shaker (60 rpm). Chromosomes were stained with 2% acetocarmine directly after pretreatment for 30 min in 0.001% colchicine solution at 14 °C during the maximum division.

Results and discussion. The most common observation was the divergency in the chromosomal complement in the

[1] A. C. Hildebrandt, A. J. Riker and B. M. Dugger, Am. J. Bot. *33*, 591 (1946).

Percentage distribution of different ploidy cells at 6 month interval (total count 150–200 cells in each determination)

Tissue age in months	*n*	2*n*	3*n*	4*n*	5*n*	6*n*	7*n*	Aneuploid cells (including hypohaploid and higher than 7*n* cells	Cells with cytological irregularities[a]
1	6.10 ± 1.86	78.09 ± 3.23	—	12.20 ± 2.55	—	—	—	3.66 ± 1.46	5.22 ± 1.73
6	7.18 ± 2.08	64.41 ± 3.87	1.31 ± 0.91	13.07 ± 2.72	—	1.31 ± 0.91	1.31 ± 0.91	11.46 ± 2.57	9.48 ± 2.36
12	2.76 ± 1.56	65.81 ± 4.53	3.70 ± 1.89	7.41 ± 2.52	—	2.78 ± 1.58	—	16.51 ± 3.57	13.66 ± 3.30
18	4.90 ± 2.13	56.86 ± 4.90	7.84 ± 2.66	10.78 ± 3.07	—	—	1.20 ± 1.07	18.52 ± 3.84	15.13 ± 3.54
24	8.51 ± 2.34	53.19 ± 4.20	6.38 ± 2.05	11.35 ± 2.61	0.71 ± 0.70	2.13 ± 1.21	3.55 ± 1.55	14.18 ± 2.93	15.24 ± 3.02
30	10.05 ± 2.24	41.89 ± 3.68	4.47 ± 1.53	18.99 ± 2.93	0.57 ± 0.56	—	3.93 ± 1.45	20.10 ± 2.99	22.98 ± 3.14
36	8.40 ± 2.54	42.86 ± 4.53	7.56 ± 2.42	13.45 ± 3.12	—	—	1.68 ± 1.17	26.04 ± 3.45	17.13 ± 3.45
42	5.33 ± 1.69	46.66[b] ± 3.77	4.00 ± 1.48	14.67 ± 2.67	—	—	6.67 ± 1.88	22.67[b] ± 3.16	20.62 ± 3.05

[a] Total percentage of cells with unequal separation of chromosomes, lagging chromosomes and bridges at anaphase and micronuclei formation at telophase. [b] Significant at $p < 0.001$.