($^3H/^{35}S = 1.92$) and 1.96 μg (5 nmMol) 7α-^{3}H-DHEA ^{35}S-sulphate-Na with 528,000 dpm ^{3}H and 271,000 dpm ^{35}S ($^3H/^{35}S = 1.95$) were incubated in duplicate with placental microsomes[5], equivalent to 250 mg of wet tissue, in 0.1 *M* phosphate buffer of pH 7.2 and in the presence of 1.5 mg $NADPH_2$, 22.6% and 17.2% of ^{3}H-activity were found in the fraction of free and conjugated phenolic steroids. Following the ion exchange chromatography of steroid conjugates on DEAE-Sephadex A-50[6] and thin layer chromatography of the steroid sulphates on silica gel G in chloroform-methanol-ammonia (20:5:0.2 v/v), on DEAE-cellulose in isopropanol-water-formic acid (65:33:2 v/v), and paper chromatography in isopropyl ether-ligroin-t-butanol-ammonia-water (5:2:3:1:9 v/v)[7], the radioactive compound with the mobility of authentic estrone sulphate (Rf = 0.17; Rf = 0.12; RT = 1.05) represented 15.9% ^{3}H of incubated androstenedione sulphate and 10.4% ^{3}H of incubated DHEA sulphate. The corresponding $^3H/^{35}S$ ratio of the isolated fractions amounted to 2.03, 2.14 and 1.98 or 2.10, 2.04 and 2.01 respectively. After cleavage of estrone sulphate by solvolysis in ethyl acetate/sulphuric acid the liberated estrone was isolated and characterized by reverse isotope dilution and purification to constant specific activity.

From these findings it becomes evident indeed that the 3,5-dienol sulphate of androstenedione can be converted biosynthetically into estrone sulphate. The yields of this biotransformation apparently exceeded those obtained by incubation of DHEA sulphate[8,9], thus favouring the concept that the biosynthesis of estrogens from DHEA sulphate may proceed via androstenedione sulphate.

Zusammenfassung. Bei Bebrütung von synthetischem 7α-^{3}H-Androst-4-en-3,17-dion-^{35}S-sulfat mit Mikrosomen aus menschlicher Placenta in Gegenwart von $NADPH_2$ wurden 15.9% des Substrats in doppelt-markiertes Östron-sulfat mit unverändertem $^3H/^{35}S$-Verhältnis umgewandelt. Da die Ausbeute vergleichsweise höher lag als bei Verwendung von 7α-^{3}H-Dehydroepiandrosteron-^{35}S-sulfat, wird angenommen, dass die Biosynthese von Östron-sulfat aus Dehydroepiandrosteron-sulfat über ein dem Androst-4-en-dion entsprechendes 3,5-Dienol-sulfat verläuft.

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Studies on the Regional Biosynthesis and Metabolism of Catecholamines in the Central Nervous System of the Monkey

Recently it was shown that norepinephrine-H^3 injected into the lateral ventricle of the rat can accumulate in the brain, and that exogenous norepinephrine introduced in this way mixes with the endogenous stores[1]. In the present study the biosynthesis and metabolism of catecholamines was investigated in specific regions of the central nervous system (CNS) of the monkey following intraventricular injection of tyrosine-C^{14} and of dopamine-H^3. Also, the tyrosine hydroxylase activity was determined in the specific regions of the CNS.

In all experiments green monkeys (*Cercopithecus sabaeus*) weighing 2.0–3.5 kg were used. The animals were injected with dopamine-1-H^3 (50 μc, 5 μg) or with tyrosine-C^{14} (25 μc, 11 μg) into both lateral ventricles of the brain by a stereotaxic technique. In experiments with dopamine-H^3 the animals were pretreated with pheniprazine (10 mg/kg i.p.) 4 h before the intraventricular injection of the labeled amine. 2 h after administration of the labeled compounds the animals were killed and the brains were removed. The labeled amines and their metabolites were isolated and determined by previously described procedures[2]. The catecholamines were absorbed on alumina and determined fluorimetrically[3,4]. Tyrosine hydroxylase activity was determined by the procedure of NAGATSU et al.[5].

Studies with tyrosine-C^{14}. Following intraventricular injection of tyrosine-C^{14} the catechols represented only a

Table I. The in vivo and in vitro formation of catecholamines from tyrosine-C^{14} in different regions of the CNS

	Catecholamines formed cpm/g tissue[a]	
	in vivo experiments	in vitro experiments[b]
Caudate nucleus	8500 ± 600	25,000 ± 1500
Putamen	400 ± 50	32,000 ± 2000
Hypothalamus	4000 ± 500	4800 ± 600
Brainstem	1500 ± 150	N.E.
Cerebellum	1050 ± 100	N.E.
Spinal cord	1000 ± 100	N.E.

[a] Each value is the mean from 3 experiments ± S.E.M. [b] The tissue homogenates were incubated with tyrosine-C^{14} for 30 min at 37 °C. N.E. = not estimated.

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Table II. Regional distribution and metabolism of dopamine-H^3

	DA $\mu g/g$	DA-H^3 $m\mu c/g$	3-MDA-H^3 $m\mu c/g$	NE-H^3 $m\mu c/g$	3-MNE-H^3 $m\mu c/g$
Caudate nucleus	4.80 ± 0.5	900 ± 100	700 ± 100	120 ± 20	15 ± 3[a]
Putamen	5.20 ± 0.6	100 ± 10	25 ± 5	N.D.	N.D.
Hypothalamus	N.E.	400 ± 50	250 ± 50	500 ± 75	50 ± 5
Brainstem	N.E.	200 ± 30	150 ± 20	300 ± 30	50 ± 5

[a] Each value is the mean of 3 experiments ± S.E.M. DA = dopamine; MDA = methoxydopamine; NE = norepinephrine; MNE = methoxynorepinephrine; N.E. = not estimated; N.D. = not detectable < 5 $m\mu c/g$.

small proportion of the total radioactivity present in different regions of the CNS. The major radioactivity of the catechols in all analyzed regions of the CNS was associated with dopamine. It is evident from the results presented in Table I that the amounts of radioactive catechols formed in the caudate nucleus were much greater than the amounts formed in other analyzed regions of the CNS. It can also be seen from the data in Table I that the tyrosine hydroxylase activity is the highest in the caudate nucleus and putamen. With the exception of the putamen, there is a close correlation in all analyzed regions of the CNS between tyrosine hydroxylase activity in vitro and the in vivo formation of catechols from tyrosine-C^{14}. The high activity of tyrosine hydroxylase in the caudate nucleus and putamen might be responsible for the high levels of dopamine in these regions of the CNS and for the high rate of dopamine formation[6].

Studies with dopamine-H^3. The distribution of dopamine-H^3 in different regions of the CNS does not entirely correspond with the distribution of endogenous dopamine (Table II). 2 h after the intraventricular injection of dopamine-H^3 the specific activity of dopamine in the caudate nucleus is much higher than in the putamen. These findings suggest that intraventricular injected dopamine-H^3 does not penetrate to the putamen to the same extent as to the caudate nucleus, and it is conceivable that the highly myelinated internal capsule represents a barrier for the penetration of the labeled amines into the putamen. The finding that norepinephrine is formed from dopamine in the caudate nucleus demonstrates that the caudate nucleus is capable of synthesizing norepinephrine. It was previously reported that the activity of the enzyme dopamine-β-hydroxylase in vitro is high in the caudate nucleus[7]; however, the present findings show that the activity in vivo is rather low.

In the hypothalamus and brain stem the major part of the radioactivity was associated with dopamine and norepinephrine. It should also be noted that large amounts of 3-methoxydopamine, but only small amounts of 3-methoxynorepinephrine, were detected in these regions of the CNS. This suggests that norepinephrine formed intraneuronally from dopamine is protected from inactivation by catechol methyl transferase. Of considerable interest is also the finding that dopamine-H^3 accumulates in the hypothalamus and brainstem. It is conceivable that dopamine accumulates in these regions in separate neurons and therefore dopamine-H^3 is not converted to norepinephrine-H^3, but it is also possible that the accumulation of dopamine is due to a slow conversion of dopamine to norepinephrine.

In confirmation with the previously reported findings[8] it was also shown that in the caudate nucleus and brainstem a very small percentage of the radioactivity is associated with epinephrine. In all the analyzed regions of the CNS some of the radioactivity was associated with the fraction which contained acidic and neutral metabolites of dopamine. From this fraction an unidentified metabolite was isolated which has the chromatographic characteristics of an amide of a higher fatty acid.

Recently the metabolism of norepinephrine-H^3 and dopamine-H^3 was investigated in the brain of rats following intraventricular injection of the labeled amines[9,10]. However, these studies did not reveal the distribution and metabolism of the labeled amines in specific areas of the basal ganglia.

The present study shows that after intraventricular administration the labeled catecholamines are taken up and are retained in some but not in all areas of the basal ganglia which contain high concentrations of endogenous catecholamines. Thus, the study of the biosynthesis and metabolism of catecholamines in the CNS by this procedure has its limitations[11].

Zusammenfassung. Nach intraventrikulärer Verabreichung von Tyrosin-C^{14} oder Dopamin-H^3 wurden die Biosynthese und der Stoffwechsel des Katecholamins in verschiedenen Regionen des ZNS bei Affen (*Cercopithecus sabaeus*) untersucht. Die grössten Katecholaminmengen wurden aus Tyrosin-C^{14} im Nucleus caudatus gebildet. Ebenso ist dort, wie im Putamen, die Tyrosin-Hydroxylase-Aktivität am grössten. Die Bildung des Noradrenalins aus Dopamin konnte im Hypothalamus, Hirnstamm, sowie im Nucleus caudatus nachgewiesen werden. Die Verteilung des radioaktiven Dopamins entspricht nicht in allen Hirnregionen der Verteilung des endogenen Dopamins.

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[12] Supported by P.H.S. Grants No. MH-02717, No. NB-5480 and No. NB-04257.