K_m are 2.27×10^{-5} M for (+)-amphetamine and 4.54×10^{-5} M for (−)-amphetamine. Furthermore, the p-hydroxylation of ^{14}C-(−)-amphetamine is more sensitive to the inducing effect of the phenobarbital treatment than that of the (+)-isomer. In the phenobarbital-treated rats, we observed an increase of apparent V_{max} (from 41.7 to 47.6 and from 71.4 to 111.1 nmoles p-hydroxyamphetamine/min mg protein for (+)- and (−)-amphetamine respectively) without effect on apparent K_m, as it is generally the case for induction phenomena.

Our results are consistent with data obtained in vivo[5,21,22] and with some results previously obtained in vitro[8,9]. Further, we could obtain under our experimental conditions a stereospecific induction of the in vitro amphetamine metabolism, (−)-amphetamine p-hydroxylation rate being greater than that observed with the (+)-isomer. Although we are unable to explain the mechanism of stereospecific induction, such a phenomenon may be of significance with regard to the pharmacological activities and interactions of these compounds.

Studies on GABA accumulation induced by γ-glutamyl-hydrazide in regions of rat brain following treatment with a tyrosine hydroxylase inhibitor and 6-hydroxy-dopamine[1]

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Summary. Reduction of DA receptor activity via depletion of DA stores does not seem to influence GABA turnover in the forebrain and in the DA cell body rich region of the midbrain.

In recent papers[2–4] evidence has been obtained that changes in GABA accumulation in brain after GABA aminotransferase inhibition using γ-glutamyl-hydrazide (GAH)[5] probably reflect changes of GABA turnover in the neuronal pool, since it has been found to be nerve impulse dependent[4]. Using this model to study changes in GABA turnover, it was possible to show that the dopamine (DA) receptor agonist apomorphine increased GABA turnover in striatum, subcortical limbic regions (rich in DA nerve terminals) and in the DA cell body rich regions of the midbrain. This increase was blocked by pretreatment with the DA receptor blocking agent pimozide. However, pimozide by itself did not cause any change of GABA turnover[2,3]. On the other hand, in dorsal neocortex and in cerebellar cortex, lacking DA cell bodies and nerve terminals, apomorphine produces no effects on GABA turnover, or a trend for a reduction (cerebellar cortex)[6], supporting the view that the increases of GABA turnover observed are due to stimulation of specific DA receptors innervated by DA terminals.

In view of this, it is of interest further to evaluate the influence of a reduction of DA receptor activation on the turnover of GABA. Therefore, it has been studied whether combined treatment with the tyrosine hydroxylase inhibitor α-methyl-tyrosine methylester (H 44/68) and the neurotoxic compound 6-hydroxy-dopamine (6-OH-DA), causing a relatively selective degeneration of catecholamine (CA) neurons in the brain[7] without affecting e.g. GABA levels[8–10], can change the GABA turnover in the nuc. caudatus, subcortical limbic regions (tuberculum olfactorium, nuc. accumbens and tractus diagonalis area) and the DA cell body rich regions of the midbrain.

Material and methods. Male specific pathogen-free Sprague-free Sprague-Dawley rats were used. Unilateral lesion of

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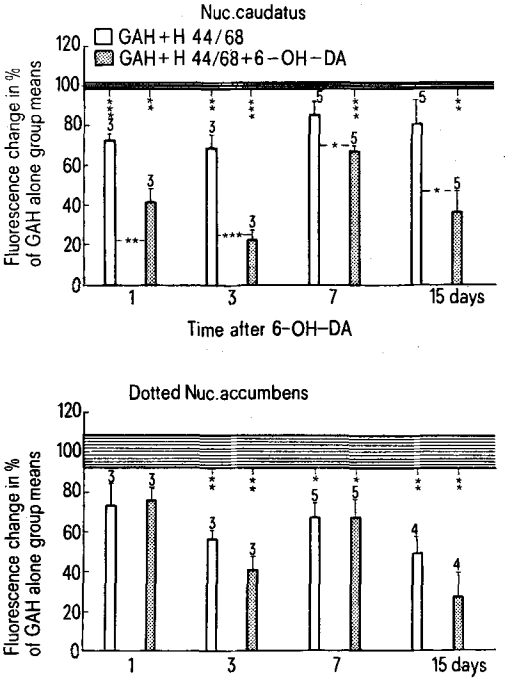

Fig. 1. Effect of 6-OH-DA and H 44/68 on DA fluorescence in the nuc. caudatus and dotted nuc. accumbens. On the x-axis days after 6-OH-DA treatment (see Materials and methods) are shown. H 44/68 (250 mg/kg) was injected i.p. 3 h 15 min and GAH (160 mg/kg) 3 h before killing. On the y-axis the DA fluorescence (means ± SEM) is shown in percent of the GAH alone group mean value. Absolute mean values for the GAH alone group expressed in arbitrary fluorescence units ± SEM were for nuc. caudatus 15.6 ± 0.2 (n = 4) and for the dotted nuc. accumbens 32 ± 2.8 (n = 4). Number of animals in parenthesis. Paired Student's t-test was used.
*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Effects of 6-hydroxy-dopamine and α-methyl-tyrosine methylester on the GAH-induced GABA accumulation in DA nerve terminal and cell body rich regions of brain

Group	Subcortical limbic area			Nuc. caudatus			DA cell body rich area of mesencephalon		
	Denervated	Innervated	$\frac{\text{Denervated}}{\text{Innervated}} \times 100$	Denervated	Innervated	$\frac{\text{Denervated}}{\text{Innervated}} \times 100$	Denervated	Innervated	$\frac{\text{Denervated}}{\text{Innervated}} \times 100$
Intact animals		100 ± 4 (4)			100 ± 7 (4)			100 ± 8 (4)	
GAH									
1 day after 6-OH-DA GAH + H 44/68	121 ± 6 (4)	128 ± 4 (4)	97 ± 3 (4)	128 ± 9 (4)	119 ± 8 (3)	110 ± 5 (3)	114 ± 8 (4)	124 ± 2 (4)	92 ± 6 (4)
3 days after 6-OH-DA GAH + H 44/68	93 ± 7 (4)	97 ± 3 (4)	96 ± 8 (4)	131 ± 10 (4)	122 ± 18 (3)	119 ± 12 (3)	124 ± 13 (4)	111 ± 5 (4)	111 ± 10 (4)
7 days after 6-OH-DA GAH + H 44/68	121 ± 5 (5)	107 ± 10 (5)	121 ± 19 (5)	119 ± 9 (5)	128 ± 7 (5)	92 ± 4 (5)	113 ± 6 (5)	132 ± 5 (5)	87 ± 4 (5)
15 days after 6-OH-DA GAH + H 44/68	113 ± 8 (5)	105 ± 18 (5)	119 ± 23 (5)	112 ± 7 (5)	116 ± 7 (5)	96 ± 1 (5)	125 ± 5 (5)	124 ± 9 (5)	102 ± 6 (5)

GAH was given i.p. in a dose of 160 mg/kg 45 min after H 44/68 (250 mg/kg, i.p.). Rats were killed 3 h after GAH treatment. Results are expressed as percent ± SE of the intact GAH treated group means. Absolute values for these groups were in μmoles/g wet tissue: 4.6 ± 0.2 for the limbic areas, 3.6 ± 0.3 for the nuc. caudatus, and 5.3 ± 0.4 for the DA cell body rich area of the mesencephalon. Absolute mean values ± SEM in these regions were for normal animals 2.8 ± 0.1, 1.6 ± 0.1 and 4.7 ± 0.4 μmoles/g wet tissue when taken in the same order as above. Number of animals in parenthesis. No statistical differences were observed according to a one way analysis of variance.

the ascending DA pathways to the forebrain was obtained by injecting 6-OH-DA (8 μg/4 μl) into the ventral tegmental area as described by Ungerstedt[7]. The rats were killed by rapid decapitation 1, 3, 7 and 15 days following the 6-OH-DA injection. Animals were injected with H 44/68 (250 mg/kg, i.p.) 3 h 15 min and with GAH (160 mg/kg, i.p.) 3 h before decapitation. GABA determinations were made in perchloric acid extracts of the various parts of the brain using the enzymatic method of Scott and Jacoby[11] as has previously been described[2]. The DA stores in nuc. caudatus, nuc. accumbens and tuberculum olfactorium were evaluated using the histochemical fluorescence method of Falck and Hillarp for the demonstration of CA. The DA fluorescence was measured with the help of a quantitative microfluorimetric analysis using a microspectrofluorograph equipped with an MPV system[12,13]. The DA fluorescence in nuc. caudatus was measured in the anterior part of the caput close to the lateral ventricle. In nuc. accumbens, the dotted DA fluorescence was measured in the area lying immediately medially of the lateral ventricle in the posterior part of the nucleus[14] and the diffuse DA fluorescence in the dorsomedial posterior part. The DA fluorescence in the tuberculum olfactorium was also measured in the posterior part in layer II close to layer I.

Results and discussion. Treatment with GAH alone did not change the DA fluorescence in the various of the forebrain studied. As seen in figure 1, the disappearance of DA fluorescence in nuc. caudatus was significantly more marked after both H 44/68 and 6-OH-DA than after H 44/68 alone. This reduction of DA fluorescence ranged from 20 to 70% of that in the GAH alone group. A significant disappearance of the DA fluorescence on the innervated side was only observed on day 1 and 3 following

Fig. 2. Correlations between degeneration of DA nerve terminals in the nuc. caudatus and in the nuc. accumbens following a 6-OH-DA injection into the ventral tegmental area. The degree of degeneration is expressed as the percentage difference between the denervated and the innervated side. On the x-axis, the degeneration of the DA terminals in the nuc. caudatus is shown. On the y-axis in Fig. 2A, the degeneration of DA terminals in the dotted accumbens is shown, and in Fig. 2B the degeneration of DA terminals in the diffuse accumbens is shown. Values from all time-intervals following 6-OH-DA injection have been used in the graph. Pearson's correlation coefficient has been calculated for the regression lines.

operation. In nuc. accumbens, however, (figure 1) there was no significant difference in DA fluorescence between the denervated and innervated side. However, a significant reduction of DA fluorescence compared with the control group was observed 3, 7 and 15 days following the 6-OH-DA injection. As seen in figure 2, there was a significant correlation between the degree of degeneration in nuc. caudatus and the degree of degeneration of dotted and diffuse DA fluorescence in the nuc. accumbens. The Pearson's product moment correlation coefficient between caudatus and dotted accumbens was 0.579 ($p<0.05$) and between caudatus and diffuse accumbens 0.582 ($p<0.05$). In contrast to this there was no correlation between the degree of degeneration of DA terminals in the nuc. caudatus and in tuberculum olfactorium ($r_{y/x} = 0.389$). The results of the GABA measurements are summarized in the table. As can be seen from the table, there was no significant difference between the innervated and denervated side regarding the degree of GAH induced GABA accumulations. Furthermore, there is no significant change in the degree of GAH-induced GABA accumulation when the GAH + H 44/68-treated groups are compared with the GAH alone group. Furthermore, when intraindividual correlations were made between the degree of DA fluorescence disappearance and the GAH-induced GABA accumulation, no significant correlations were found when using the Pearson's product moment correlation coefficient.

The present findings show that, inspite of a depletion of DA stores with the help of combined treatment with 6-OH-DA and the tyrosine hydroxylase inhibitor H 44/68, it was not possible to change GABA accumulation in the nuc. caudatus, in subcortical limbic areas and in the DA cell body rich region of the midbrain. These findings agree with the results obtained using the DA receptor blocking agent pimozide. Thus, treatment with pimozide was found not to change GABA accumulation in the 3 regions mentioned above (see[2] and unpublished data). In contrast, when drugs are given such as apomorphine which increases DA receptor activity, a clearcut increase in GABA accumulation is observed in these 3 regions[2,3]. Thus, it seems as if increases of DA receptor activity will result in increases of GABA accumulation, but only in DA nerve terminal and cell body regions[6]. A reduction of DA receptor activity, on the other hand, will have less influence on GABA accumulation in DA nerve terminal and cell body rich regions. These studies will now be continued by making lesions which produce a far more complete degeneration of the DA systems and by the use of other types of DA receptor blocking agents.

It should be mentioned that the degree of 6-OH-DA-induced disappearance of DA stores in the nuc. caudatus and in the 2 parts of the nuc. accumbens studied was significantly correlated, whereas this was not true for the disappearance of DA stores in nuc. caudatus and tuberculum olfactorium. These findings may suggest that the DA pathways to the nuc. caudatus and nuc. accumbens run close together in the ventral tegmental area.

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Immunohistochemical demonstration of an SRIF-like system in the brain of the reptile: Lacerta muralis Laur.[1]

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Summary. The distribution of an SRIF-like substance in the brain of normal lizards (Lacerta muralis Laur.) has been determined. SRIF is shown to be present in neural cell bodies in the hypothalamic paraventricular nucleus, the hypothalamo-hypophysial tractus and the median eminence.

In the Poïkilotherms, cytoimmunological research on polypeptidic neurosecretion essentially concerns gonadotropic LHRH hormone[2–5]. We only know of one study on the SRIF factor (somatostatin-release-inhibiting hormone) in the Amphibian[6]. One of us (Dubois) prepared antibodies against this substance and checked immunoreactive specificity towards the antigene. In this study SRIF antisera were used to trace the SRIF-like system in the brain of a reptile: Lacerta muralis, by immunofluoroscence.

Techniques. We used SRIF and neurophysin antisera as described in earlier publications[6–8]. Indirect immunocytological reactions were examined in 12 lizard brains fixed with Bouin Holland sublimate without acetic acid, dehydrated and embedded in paraffin and then cut into 7 µm sections along the frontal, sagittal and transversal planes. Controls were carried out on contiguous sections treated respectively with non-inhibited antibodies, and with the same serum saturated with 200 µg of antigen per ml of undiluted serum. SRIF antiserum is then completely inhibited as regards the antigen present in the brain, and no immunofluorescent reaction is detected on the sections. In addition, inhibition reactions with other peptides present in the brain and median eminence

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