bradykinin-induced release of prostaglandin-like material[12].

Zusammenfassung. GP 45 840, das Natriumsalz der [*o*-[(2,6-Dichlorphenyl)-amino]-phenyl]-essigsäure, besitzt pharmakodynamisch in verschiedenen Testsystemen am Tier eine ausgeprägte anti-inflammatorische, antinociceptive und antipyretische Aktivität. Das Präparat zeigt eine höhere Wirksamkeit als Phenylbutazon und ist ebenso aktiv wie Indomethacin; es übertrifft in seiner akuten therapeutischen Breite die beiden Vergleichspräparate.

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Effect of Spreading Depression on Electrical Activity and Dopamine Turnover in the Striatum of Rats

Epidural application of hyperosmolar KCl solutions on one hemisphere of the rat brain produces depolarization which spreads to the whole cortex[1-4] and the striatum of the ipsilateral side[5] (spreading depression, SD). The SD is associated with an increase in the turnover of brain stem norepinephrine[6] and of striatal dopamine (DA)[7,8]. However, the changes in electrical activity as well as the correlation between biochemical and neurophysiological events during a prolonged SD are not well known. Therefore, in the present paper the effect of epidural application of KCl during 1 h on the electrical activity and the DA turnover in the basal ganglia have been investigated.

Methods. Male albino rats of Wistar origin (Füllinsdorf), weighing 200–250 g, were anaesthetized with Thiogenal®. In a first group of 15 animals, 2 screw electrodes were implanted on each parietal bone 2 and 4 mm, respectively, from the sagittal line and 5 mm anterior to the lambda. Two concentric electrodes (0.3 mm in diameter) were placed in each corpus striatum (coordinates: A = 8.2, L = 3.0, V = +6.0)[9]. An electrolytic lesion made at the end of each experiment facilitated the histological control. All bipolar derivations were connected to a Grass polygraph (model 7B) through a shielded cable attached to an Amphenol strip plug cemented to the skull. A sealed plastic cannula containing a cotton plug moistened with 0.9% NaCl was placed over the dura of the right hemisphere exposed by a skull opening 2 mm anterior and 3 mm lateral to the lambda. A second group of 92 animals in which only the plastic cannula was implanted served for the biochemical assays.

Twenty to 24 h after the operation, the cotton plug in the plastic cannula was replaced by another one impregnated with KCl (25%) or NaCl (20%) which in the first group of rats was again replaced after 1 h by physiological saline. The electrical activity was recorded during the 30 min prior to and 4 h following application of KCl (25%) or NaCl (20%), the rats being kept in a large bell-shaped glass jar under normal laboratory conditions.

In the second group, some of the rats were decapitated 30 or 60 min after epidural application of the concentrated electrolyte solutions, whereas in others KCl (25%) or NaCl (20%) was again replaced after 1 h by 0.9% NaCl. The animals were then sacrificed at various time intervals. Rats killed 20–24 h after the operation without epidural treatment served as controls.

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Effect of epidural application of NaCl or KCl on the content of endogenous homovanillic acid (HVA) in brain of rats

Min after electrolyte application	NaCl, 20%		KCl, 25%	
	L	R	L	R
0	0.092 ± 0.006	0.096 ± 0.005		
30	0.098 ± 0.007	0.100 ± 0.008	0.112 ± 0.003	0.149[a] ± 0.007
60	0.098 ± 0.004	0.106 ± 0.005	0.108 ± 0.005	0.170[a] ± 0.008
120	0.100 ± 0.006	0.109 ± 0.006	0.103 ± 0.003	0.146[a] ± 0.003
180	0.100 ± 0.005	0.100 ± 0.004	0.103 ± 0.004	0.128[a] ± 0.006
240	0.105 ± 0.010	0.112 ± 0.005	0.093 ± 0.008	0.098 ± 0.009

20% NaCl or 25% KCl was applied on the dura of the right cerebral cortex at time 0. Some of the rats were decapitated 30 or 60 min later, whereas in others the concentrated electrolytes were replaced after 60 min by 0.9% NaCl, and sacrifice followed at the time intervals indicated. Operated animals without epidural treatment served as controls (= values for time 0). The concentration of HVA was determined in 2 pooled left (L) or right (R) cerebral hemispheres and is expressed in µg/g wet weight. The values represent means with SEM of all determinations in 2–3 experiments, each performed with 4 rats per group. [a] $p < 0.01$ compared to the corresponding left hemisphere. All the other values of the right hemisphere are not significantly different ($p > 0.05$) from those of the corresponding left hemisphere.

The determinations of homovanillic acid (HVA) and DA were carried out in two pooled left or right cerebral hemispheres (halves of the brain, without cerebellum, pons and medulla oblongata). HVA was extracted with n-butylacetate[10] and measured fluorimetrically[11]; DA was also assayed by a fluorimetric method[12].

Results. 1. Controls. During the 30 min preceding the application of 25% KCl, the records of the electrical activity from cortex and striatum of both hemispheres showed an alternation of patterns of slow-wave sleep (SWS) and wakefulness, with a prevalence of SWS. In the periods of wakefulness, the records were characterized by a rapid activity with a wave frequency of 25–35/sec and 40–60 μV in amplitude (Figure). During SWS, after the initial spindling, slow waves of very high voltage, up to 450 μV, with a frequency of 4–6/sec appeared.

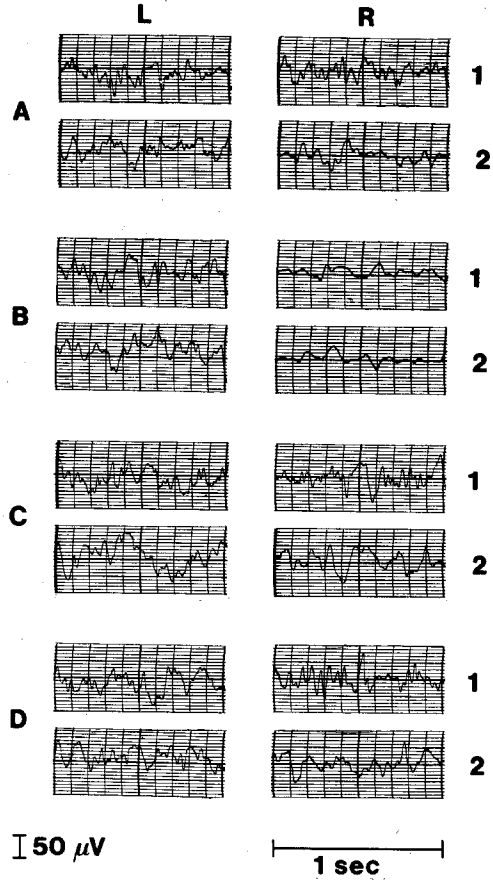
2. Epidural KCl or NaCl. Application of 25% KCl on the right dura produced within 1–2 min in the right but not in the contralateral cortex a marked reduction of the frequency of the electrical activity (6–7/sec) with disappearance of the faster rhythms and decrease of the amplitude (10–20 μV) of the slow waves (Figure). These changes appeared, with a latency of about 3 min, also in the striatum ispilateral to the KCl application in 13 out of the 15 animals investigated. Concomitantly with the electroencephalographic changes the concentration of HVA markedly increased in the ispsilateral but not in the controlateral hemisphere. This rise was maximal 1 h after KCl application (Table).

Replacement of KCl after 1 h by 0.9% NaCl resulted in a normalization of the electrical activity within 30 min (Figure). The HVA concentration of the ipsilateral side was, however, still elevated 2 h after removal of KCl, and control levels were reached only after 3 h (Table). The DA concentration did not change in either hemisphere at any time.

When 20% NaCl instead of 25% KCl was applied, neither electrophysiological (Figure) nor biochemical changes were observed.

Discussion. The present results indicate that prolonged epidural application of KCl (1 h) causes electrophysiological changes similar to those found during short-term (1–5 min) KCl exposure[2–5]. They last for the period of application of KCl and disappear rapidly after its removal. The electrical changes possibly correspond to a long-lasting but reversible functional inactivation of the striatum[1–5,13–17]. Experiments with drugs confirm this view. Thus, after unilateral application of KCl, apomorphine, D-amphetamine and L-dopa cause the rats to rotate towards the side of SD[18]. This behaviour is comparable to that induced by these drugs in rats with one striatum coagulated[19] and probably indicates that only the DA receptors in the intact but not in the depolarized striatum react to pharmacological stimulation.

Concomitantly with the onset of changes in the electrical activity, the striatal HVA concentration of the ipsilateral hemisphere starts rising, whereas DA remains unaltered. This increase in HVA probably results from an enhanced turnover of DA. Thus, inhibition of HVA outflow from the brain is unlikely since 5-hydroxyindoleacetic acid (which is transported by the same mechanism as HVA[20]) was not changed by KCl. In addition, KCl but not NaCl significantly accelerated the α-methyl-*p*-tyrosine-induced disappearance of DA in the ipsilateral hemisphere[7,8]. The mechanism of the enhanced DA turnover is, hovever, still unknown. It might be speculated that depolarization inactivates a cortico-nigral or a striato-nigral inhibitory projection ot the DA cell bodies. This would lead to an increase in the activity of dopaminergic neurons and thereby to an enhanced DA turnover. The persistence of an increase of HVA after normalization of the EEG pattern does not contradict the assumption of a possible causal relationship between decreased electrical activity and enhanced DA turnover. Thus, the clearance of HVA from the brain probably takes some time[21] so that increased levels of the acid may persist for a limited period of time after normalization of DA turnover.



Electroencephalographic activity of the cerebral cortex and striatum. A) 30 min before KCl application; B) 30 min after KCl application; C) 30 min after removal of KCl; D) 30 min after application of 20% NaCl. The electrolyte solutions were applied on the dura of the right cerebral hemisphere. 1. cortex; 2. striatum; L, left; R, right hemisphere.

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Zusammenfassung. Während einstündiger epiduraler Applikation von 25% KCl zeigte sich bei Ratten eine Abnahme der elektrischen Aktivität im homolateralen Cortex und Striatum (spreading depression), die nach Entfernung des KCl reversibel war. Mit dem Auftreten von EEG-Veränderungen erfolgte im Gehirn auch ein reversibler Anstieg der Homovanillinsäure, nicht aber des Dopamins (DA), wahrscheinlich als Ausdruck eines gesteigerten DA-Umsatzes. Es wird geschlossen, dass während unilateraler epiduraler KCl-Applikation eine Inaktivierung des homolateralen Striatums besteht, welche möglicherweise durch Desaktivation einer inhibitorischen cortico- und/oder striato-nigralen Bahn zu einer Erhöhung des DA-Umsatzes führt,

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Effect of Long-Term Fenfluramine Treatment on Drug-Metabolism in Rat

Fenfluramine, N-ethyl-α-methyl-3-(trifluoromethyl)-phenethylamine, is structurally related to amphetamine, has anorexic activity, but has been claimed to be devoid of the central nervous system stimulating activity[1]. Its acute and chronic toxicity have been evaluated in various species[2]. Fenfluramine is metabolized by dealkylation and probably by sidechain oxidation. The main metabolite of fenfluramine in human urine is m-trifluoro-methyl-hippuric acid[3]. We have been interested to know whether long-term fenfluramine treatment interfers, and to what extent, with the drug metabolizing enzymes of rat liver.

Materials and methods. Male Sprague-Dawley rats weighing 250–300 g were divided into 8 groups of 5 each. The animals received food and water ad libitum. Once a day they were given by stomach tube fenfluramine hydrochloride aqueous solution either 10 mg/kg (2 groups), 25 mg/kg (2 groups) or 50 mg/kg (2 groups). The 2 control groups received only water. One group of each dose level were decapitated 3 weeks and the others 8 weeks after the first dose. Livers were removed and homogenized in 4 volumes of 0.1*M* phosphate buffer of pH 7.4. The 105,000 × *g* microsomal fraction was used for the determinations of the activities of benzpyrene hydroxylase (BPH)[4], N-methyl-aniline demethylase (MAD)[5] and uridine diphosphoglucuronyl transferase (UDPGT)[6]. The cytochromes P-450 and b_5 were quantitated as described by OMURA and SATO[7]. Significance of difference from controls was calculated using the Student's *t*-test.

Results and discussion. In contrast to numerous amphetaminelike drugs, fenfluramine has been reported to produce greater loss of weight and fewer side-effects[8]. In our studies we found a slight reduction in food consumption and a retardation of weight gain which, however, was significant ($p < 0.05$) only in the group receiving the largest dose (50 mg/kg) of fenfluramine for 8 weeks. The animals receiving 25 or 50 mg/kg of fenfluramine daily p.o. showed hyperexcitability at the beginning of the treatment. This is contradictory to many other studies[9–11], which indicate rather a sedative effect, but is in agreement with those of EVERETT et al.[12] who observed that a mean dose of 6.1 mg/kg of fenfluramine given i.p. increased substantially the motor activity in rats. Interestingly enough, it has been shown by COSTA et al.[13] that fenfluramine possesses similar types of action on brain amines to amphetamine, e.g. it depletes the brain noradrenaline content.

In both our trials the doses of 25 and 50 mg/kg caused a significant increase in the relative liver weights shown in Table I, but in histological examinations no morphological changes could be found. The increase in the relative liver weight is common to many inducing agents because they produce proliferation of the endoplasmic reticulum and increase the protein content of the liver. As can be seen in Table II, both higher doses of fenfluramine really stimulated the drug metabolizing capacity of the rat liver microsomes. This effect is most clearly seen as an enhancement of MAD activity and the amount of Cytochrome P-450. The inducing effect was already maximal after 3 weeks' treatment. Fenfluramine causes a Type I differential spectrum when binding with Cytochrome P-450 (unpublished observation) which indicates that its metabolism may be accelerated by a phenobarbital-type of inducing agents. It has recently been shown that fenfluramine may also stimulate its own metabolism[14], which possibly explains the disappearance of the hyperexcitability mentioned and the lack of toxic symptoms after 8 weeks' treatment with the dose up to about half of the LD 50, which, according to GILBERT et al.[2], is 126 mg/kg.

Table I. Relative liver weights of rats receiving 10, 25 and 50 mg/kg of fenfluramine hydrochloride for 3 and 8 weeks

Fenfluramine (mg/kg)	3 weeks treatment	8 weeks treatment
Controls	2.69 ± 0.09	2.56 ± 0.19
10	2.91 ± 0.33	2.80 ± 0.23
25	3.24 ± 0.39[a]	3.33 ± 0.26[a]
50	3.61 ± 0.33[b]	3.47 ± 0.08[b]

The values are expressed as g liver/100 g body weight ± SD. Significance of difference from controls shown by [a] $< p$ 0.01; [b] $< p$ 0.001.

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