bioelectric fields among other things for prey detection. On the other hand, the hydroelectric fields could be of significance in orientation.

To test the first supposition we investigated how catfish reacted to an artificial plastic prey with imitated electric field. For this purpose 4 catfish were fed daily with 4 larvae of *Xenopus laevis*, the clawed toad. Two other catfish were fed daily with 4 pieces of meat. After 1 week 2 plastic dummies were simultaneously introduced in the fish tank during feeding. One of these dummies generated an artificial electric field that corresponded with the natural electric field of a *Xenopus* larva. These natural electric fields, which showed a strong dipole character (mouth +with respect to gill slits), were earlier recorded on magnetic tape and could be reproduced by feeding the taped signal via an isolation unit and a voltage-to-current-converter to 2 silver thread electrodes fixed at those sites of the plastic dummies where mouth and gill slits were supposed to be. The electric field so reproduced corresponded sufficiently well to the original field of the real *Xenopus* larva. The responses of the catfish to each of these plastic larvae with (X $\sim$) and without (Xo) electric field were protocolled as 1. *Swallowing*, when the fish after having approached the dummy to about 5 cm jerked its mouth heavily in the direction of the prey and tried to devour it; 2. *Interest*, when the fish nibbled quietly at the imitated prey; 3. *No reaction*, when it neither swallowed nor nibbled; 4. *Flight*, when the fish increased its swimming speed in the direction of the bottom, after having reached the vicinity of the dummy. The reactions of fish fed with *Xenopus* are represented in Table I; those of fish fed with meat in Table II.

The chi-square test applied to the material of Table I gave a significance smaller than $^1/_2\%$ ($X^2 = 55$, $n = 3$) for differences between responses to X $\sim$ and Xo, i.e. electric and non-electric dummies. Catfish fed with meat showed not that great difference. For these animals the chi-square test gave no significant differences between responses to X $\sim$ and Xo ($X^2 = 4.0$, $n = 3$, $p > 5\%$). No statistics are needed to see that the 'swallowing'-reactions of these fish to X $\sim$ are less frequent than those of fish fed with *Xenopus* larvae. These results show clearly that *Ictalurus* can detect such dummies – if it recognizes them as food – by means of their imitated bioelectric fields. This is a strong indication for the use of electric fields in prey detection.

To test the second hypothesis, orientation in electric fields, catfish were placed in different electric stimulus situations, i.e. electric fields of different forms, dimensions, strengths and durations. As the results are rather incomplete yet, we will mention only the most conspicuous traits in the spontaneous behaviour of the fish. In DC-fields with strengths corresponding with the natural hydroelectric fields, *Ictalurus* proved to orientate itself parallel with the field lines. Further the fish mostly dwelled in those areas where current density was lowest.

Both kinds of experiments mentioned above indicate the importance of natural bioelectric and hydroelectric fields to the electrosensitive catfish. Other experiments are in progress.

Zusammenfassung. Verhaltensversuche, bei denen die Reaktionen des Zwergwelses (*Ictalurus nebulosus* LeS.) auf künstlich wiedergegebene pseudo-bioelektrische und -hydroelektrische Felder registriert wurden, ergaben, dass *Ictalurus* beim Beutefang die von der Beute hervorgebrachten elektrischen Felder benützen kann und dass in hydroelektrischen Feldern spontan Orientierungsverhalten auftritt.

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Interaction Between β-Adrenergic Stimulant and Phosphodiesterase Inhibiting Drugs on the Bronchial Muscle

Recent reports show that bronchodilator effects can be achieved by adenylcyclase stimulation, phosphodiesterase inhibition and even by direct injection of 3′5′AMP[1,2]. We therefore considered it would be of interest, both from a theoretical and a practical point of view, to investigate the types of interaction existing between salbutamol, a selective, long-acting β-stimulant agent[3–5], theophilline and dibutyrril-3′5′AMP, a lipophilic derivative of the cyclic nucleotide.

Assuming that salbutamol acted through adenylcyclase stimulation, synergism could be expected between this drug and theophilline, known to inhibit phosphodiesterases, or dibutyrril-3′5′AMP which is not a substrate for these enzymes[6] and even inhibits them at concentrations as low as 100 μM[7].

Experiments were carried out on urethane-anaesthetized guinea-pigs, according to the slightly modified[2] method of KONZETT and RÖSSLER[8], and on guinea-pig tracheal chain, set up according to AKASU[9]. In both experimental conditions, histamine was chosen as the bronchoconstrictor agent. Its dose/effect curves were determined before and in the presence of the 3 drugs given at different dosage.

The ratio between histamine equiactive doses before and after treatment was calculated; this value $\times 100$, referred to as % responsiveness, was inversely related to drug dosage and allowed us to calculate ID_{50} or IC_{50} (i.e., drug doses or concentrations able to halve the responsiveness of the preparations to the agonist). The results are summarized in Tables I and II. The potency of salbutamol was many times greater than that of theophylline and dibutyrril-3′5′AMP, but a definite potency ratio could not be calculated because of the different slopes of the dose/effect curves.

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Table I. Antagonism exerted by salbutamol, theophylline, dibutyrril-3′5′ AMP and their associations (i.v. injection) against histamine bronchospasm in the guinea-pig

Treatment	Dose/Kg	No. of experiments	Responsiveness ± S.E. (%)	ED_{50}	b
Salbutamol	1 μg	5	81 ± 3.6		
	3 μg	6	59 ± 6.1	7.7 μg/kg	−31
	10 μg	5	47 ± 6.8		
	30 μg	6	33 ± 2.5		
Theophylline	5 mg	9	80 ± 5.0		
	10 mg	5	65 ± 7.0	17.4 mg/kg	−57
	50 mg	5	27 ± 3.0		
Dibutyrril-3′5′ AMP	5 mg	5	87 ± 5.4		
	10 mg	5	68 ± 5.0	32 mg/kg	−42.2
	30 mg	5	54 ± 3.1		
Theophylline plus salbutamol	10 mg + 2 μg	6	45 ± 2.0	—	—
Dibutyrril-3′5′ AMP plus salbutamol	10 mg + 2 μg	6	49 ± 7.0	—	—

In order to ascertain the type of interaction in vivo, between a β-adrenergic stimulant and a phosphodiesterase inhibiting drug and 3′5′AMP, salbutamol 2 μg/kg was administered i.v. immediately after a nearly equiactive i.v. dose of theophylline (10 mg/kg) or of dibutyrril-3′5′AMP (10 mg/kg).

The reduction in the bronchial response to histamine caused by the 2 pairs of drugs was greater than that expected from simple summation of the effects. In fact, the dose/effect curves (Table I) showed that salbutamol, theophylline and dibutyrril-3′5′AMP, given separately at double dosage, caused a responsiveness of 60,49 and 59% respectively. The % responsiveness predictable from simple summation was about 55% for salbutamol plus theophylline and about 60% for salbutamol plus dibutyrril-3′5′AMP, higher than that actually found. Interaction was also examined in vitro by determining the IC_{50} of salbutamol in the presence of theophylline 5 μg/ml or dibutyrril-3′5′AMP 50 μg/ml. In this case, the sensitivity of histamine shown by theophylline or dibutyrril-3′5′AMP treated preparations was taken as 100%. As summarized in Table II, theophylline and dibutyrril-3′5′AMP significantly reduced the IC_{50} of salbutamol, as compared with the IC_{50} found in normal preparations.

Concluding, evidence was reached that both drugs potentiated the antibronchospastic effect of the sympathomimetic amine, in vivo and in vitro. The synergism can be accounted for by an increase of 3′5′AMP intracellular

Table II. Antagonism exerted by salbutamol, theophylline, dibutyrril-3′5′ AMP and their associations against histamine in the guinea-pig tracheal chain

Treatment	Concentration (g/ml)	No. of experiments	Responsiveness ± S.E. (%)	ED_{50} (g/ml)	b
Salbutamol	3×10^{-10}	4	86 ± 8.0		
	1×10^{-9}	6	54 ± 4.5	1.21×10^{-9}	−58
	3×10^{-9}	6	28 ± 3.7		
	1×10^{-8}	6	12 ± 2.0		
Theophylline	5×10^{-6}	5	95 ± 5.0		
	1×10^{-5}	5	61 ± 7.2	1.35×10^{-5}	−103
	2×10^{-5}	5	32 ± 7.7		
	5×10^{-5}	5	11 ± 2.7		
Dibutyrril-3′5′ AMP	5×10^{-5}	5	80 ± 10		
	1×10^{-4}	6	46 ± 9.5	9.32×10^{-5}	−99.8
	2×10^{-4}	5	19 ± 2.7		
Salbutamol in the presence of theophylline (5×10^{-6} g/ml)	3×10^{-10}	5	57 ± 8.1		
	1×10^{-9}	7	39 ± 4.2	4.6×10^{-10} [a]	−37.9
	3×10^{-9}	8	19 ± 2.5		
Salbutamol in the presence of dibutyrril-3′5′ AMP (5×10^{-5}g/ml)	3×10^{-10}	5	51 ± 6.0		
	1×10^{-9}	6	30 ± 4.4	3.2×10^{-10} [a]	−38
	3×10^{-9}	5	15 ± 3.7		

[a] Statistically different from the control group, salbutamol alone, P 0.02.

levels brought about by different mechanisms. Previous experiments carried out on adipose tissue and heart[10,11] stated that drugs able to stimulate adenylcyclases act synergically with phosphodiesterase inhibitors. The potentiation found by us between salbutamol and theophylline extends such a statement to the bronchial smooth muscle and indirectly confirms that salbutamol behaves as an adenylcyclase stimulating agent.
On the other hand, the finding that potentiation also exists between salbutamol and dibutyrril-3'5'AMP suggests that the phosphodiesterase inhibiting properties of the latter compound play an important role. Thus, the assumption that it acts only as an easily permeable 3'5'AMP must be regarded with some criticism.

Riassunto. È stato dimostrato che la contemporanea stimolazione delle adenilciclasi, realizzata con salbutamolo, e la inibizione delle fosfodiesterasi prodotta da teofillina esercitano effetti broncodilatatori sinergici. Il potenziamento osservato anche fra salbutamolo e dibutirril-3'5'AMP è stato riferito alla inibizione delle fosfodiesterasi determinata da quest'ultima sostanza.

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Saturable Transport of Amphetamine Across the Blood-Brain Barrier[1]

The rapid onset of CNS stimulation after amphetamine administration is usually ascribed to free diffusion of its nonionized, lipophilic form across the blood-brain barrier (BBB)[2]. However, amphetamine has a pKa of 9.9[3]; at an arterial pH of 7.4, about 99.7% of the plasma amphetamine concentration would be protonated and lipophobic. This disparity between predicted effects (slow rate of barrier permeability) and observed effects (abrupt psychomotor changes and stereotyped behavior) suggests that some portion of the predominantly ionized form of amphetamine enters the brain from the circulation by a transport mechanism. This in vivo study describes some aspects of D-amphetamine uptake across the BBB which might explain the onset discrepancy, and demonstrates for amphetamine uptake two hallmarks of carrier-mediated transport: saturability and competitive inhibition.

Methods. The penetration of ^{14}C-D-amphetamine sulfate into brain was studied with the internal tritiated water technique described by OLDENDORF[4]. Male Sprague-Dawley rats, 350–400 g, were anesthetized with 45 mg/kg i.p. pentobarbital sodium and prepared for intracarotid injection. A single bolus (constant volume of 0.2 ml) was injected into the common carotid of each rat without impeding the arterial blood flow. The solution consisted of 0.25 μCi ^{14}C-D-amphetamine sulfate (s.a. 15.5 mC/m*M*, Schwarz/Mann), 0.25 μCi ^{3}H-water (s.a. 0.25 mC/g, New

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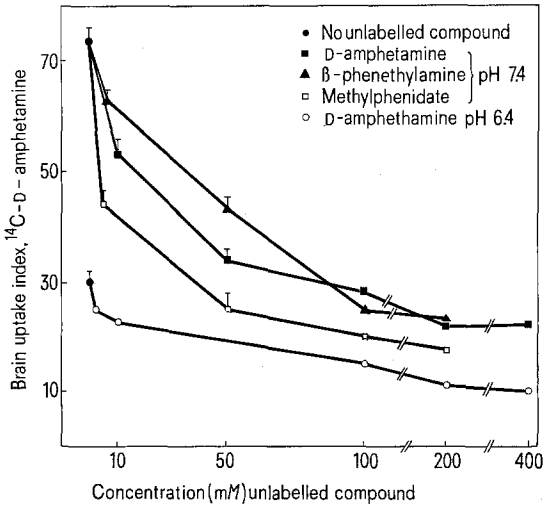

Fig. 1. The effects of increasing concentrations of various compounds on the uptake of ^{14}C-D-amphetamine sulfate into rat brain. All compounds were injected as a bolus (0.2 ml) into the carotid artery. Standard errors of the means are given for those values which represent 3 or more animals; other points are from individual rat brains.

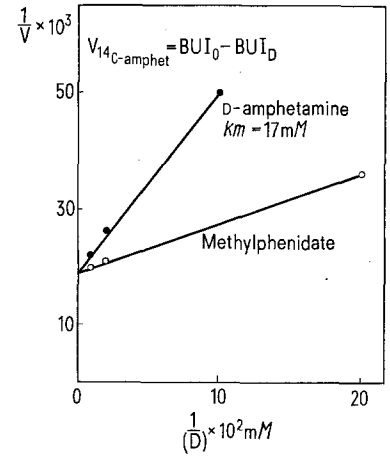

Fig. 2. Lineweaver-Burk plot of the effects of increasing concentrations of unlabelled D-amphetamine and methylphenidate on the uptake of a small dose (1 μg/kg) of ^{14}C-D-amphetamine into rat brain. Abscissa: reciprocal of the concentration of unlabelled drug (D). Ordinate: reciprocal of the differences in uptake of labelled amphetamine in the presence of specific concentrations of D.