samples from the same animal, are larger than the variations between 4 different artificial CSF preparations. It seems, therefore, that the artificial CSF could be used for studies of diffusion to and from the CSF. No ACh could be detected in all experiments in which eserine was not added.

Table II shows the effects of OTMN when added to the fluid during perfusion of the cerebral ventricles. The increase in ACh content without OTMN reached 168% after 2 h. However, when OTMN was added, after 45 min, it reached 610%. The values are given as percentages of the initial content so as partly to overcome the individual variations. The control values obtained with eserine are similar to those obtained with neostiqmine[9] and tetraethylpyrophosphate (TEPP)[5,10].

Table II. ACh collected (percentage of initial values) in subsequent samples of perfusion of the cerebral CSF containing spaces

Sample	a)	b)
1	100	100
2	157	187
3	157	107
		→
4	134	110
5	135	187
6	243	402
7	162	526
8	168	610

Each value represents the mean of 5 experiments. In controls (a) only eserine was added to the artificial CSF. OTMN was added in (b) to the perfusion fluid after 45 min of perfusion (arrow).

Table III. ACh collected (percentage of initial value) in subsequent samples of perfusion of spinal subarachnoid space

Sample	a)	b)	c)	d)
1/1	100	100	100	100
2/1	76	55	94	34
3/1	185	61	189	32
		→	→	→
4/1	274	53	99	46
5/1	190	602	172	969
6/1	190	434	281	803
				→
7/1	262	362	629	316
8/1	110	466	984	143

Each value represents the mean of 5 experiments. The controls (a) contained only eserine. OTMN was added (arrow) either by i.v. injections (b) or in the perfusion fluid (c), and in (d) Bay Va 1470 was injected (lower arrow) after the addition of OTMN.

Table III shows the effect of OTMN given both i.v. and in the fluid during perfusion of the spinal subarachnoid space. When only eserine was added, the increase in ACh content reached 110%. By i.v. injections of OTMN the amount of ACh increased by 466%, whereas by perfusion it increased by 984%. When Bay Va 1470 was injected 60 min after OTMN, a rapid decrease in ACh content was noted.

OTMN is known to increase ACh content in the brain. The present experiments show that the substance is also released from the nervous tissue. During perfusion, in which the OTMN comes closer to the nervous tissue, smaller amounts produced a larger increase in ACh output.

Bay Va 1470 is suggested as an inhibitor for ACh[11]. The present experiments, indeed, show that it causes a rapid decrease in ACh release from the CNS. Preliminary experiments in EEG, carried out in our laboratory, show that OTMN seems to lower the amplitude and raise the frequency, whereas Bay Va 1470 raises the amplitude and lowers the frequency. This is consistent with the amount of ACh obtained[12].

Zusammenfassung. Perfusion der Liquorräume im Nervensystem der Katze zeigte bei Zusatz von Oxotremorin eine Steigerung des Acetylcholingehaltes in der Perfusionsflüssigkeit. Die Wirkung von OTMN war bei der Verabreichung mit der Perfusionsflüssigkeit erheblich stärker als bei i.v. Injektion. Bay Va 1470 reduzierte hingegen wesentlich die Freisetzung von ACh.

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Prostaglandin Feedback Mechanism Limits Vasoconstrictor Action of Norepinephrine in Perfused Rabbit Ear

Prostaglandins (PG) are released by vasoconstrictors infused into the kidney[1–4] or into the spleen[5], and by sympathetic stimulation of renal[6] and splenic[7] nerves. PG release represents fresh biosynthesis, since it is blocked by indomethacin or by other inhibitors of PG biosynthesis[8].

It is not clear to what extent, if at all, the vascular wall participates in the generation of PG, which are detected in the outflow from organs treated with a vasoconstrictor, although a possibility of an intramural generation of PG by contracting blood vessels has been proposed[9,10].

Here we report that vasoconstriction induced by norepinephrine (NE) in the perfused rabbit ear is accompanied by a release of PGE-like substance, which attenuates the persistence of the pressor response to NE. Perfused rabbit ear, unlike perfused spleen or kidney, has little chance to produce PG from other sources than from contracting blood vessels.

Materials and methods. Isolated rabbit ears[11] were perfused through the central artery with Tyrode's solution, which contained 1% of dextrane, mol wt. 40,000. The perfusion fluid (37 °C) was oxygenated and pumped at a steady rate of 2–3 ml/min to maintain the perfusion pressure at a level of 30–50 mm Hg. The effluent from the ear superfused in cascade the assay tissues: a chick rectum, a rat stomach strip and a rat colon. Combined antagonists[12] were added to the effluent to make the assay more specific for PG. Final rate of superfusion was 4 ml/min, and final concentrations of antagonists (μg/ml) were: diphenhydramine 5, atropine 2, methysergide 0.1, phenoxybenzamine 0.2, propranolol 0.1, indomethacin 3. Sites of infusions of indomethacin, phenoxybenzamine or propranolol could be changed separately, and then one of the drugs was passed across the ear vessels (Figure). The initial load on each tissue was 2–3 g, and their movements were recorded with auxotonic Paton's levers at the gear 1:8. Perfusion pressure was measured by an one-arm mercury manometer.

For sake of calibration, known quantities of PGE_1, PGE_2, $PGF_{2\alpha}$ and NE were infused directly over the assay organs. PGE_1 and PGE_2 contracted all assay organs at a threshold concentration 0.5–1.0 ng/ml, while $PGF_{2\alpha}$ contracted only the rat colon. The chick rectum was insensitive to $PGF_{2\alpha}$ and a number of biologically active amines and peptides, which were tested at a concentration 5 ng/ml. NE at a concentration 100–300 ng/ml relaxed only the rat stomach strip.

Results and discussion. When NE was infused intra-arterially at a rate 300 ng/ml/min during a period of 6 min, there was observed a gradual decline of the pressor

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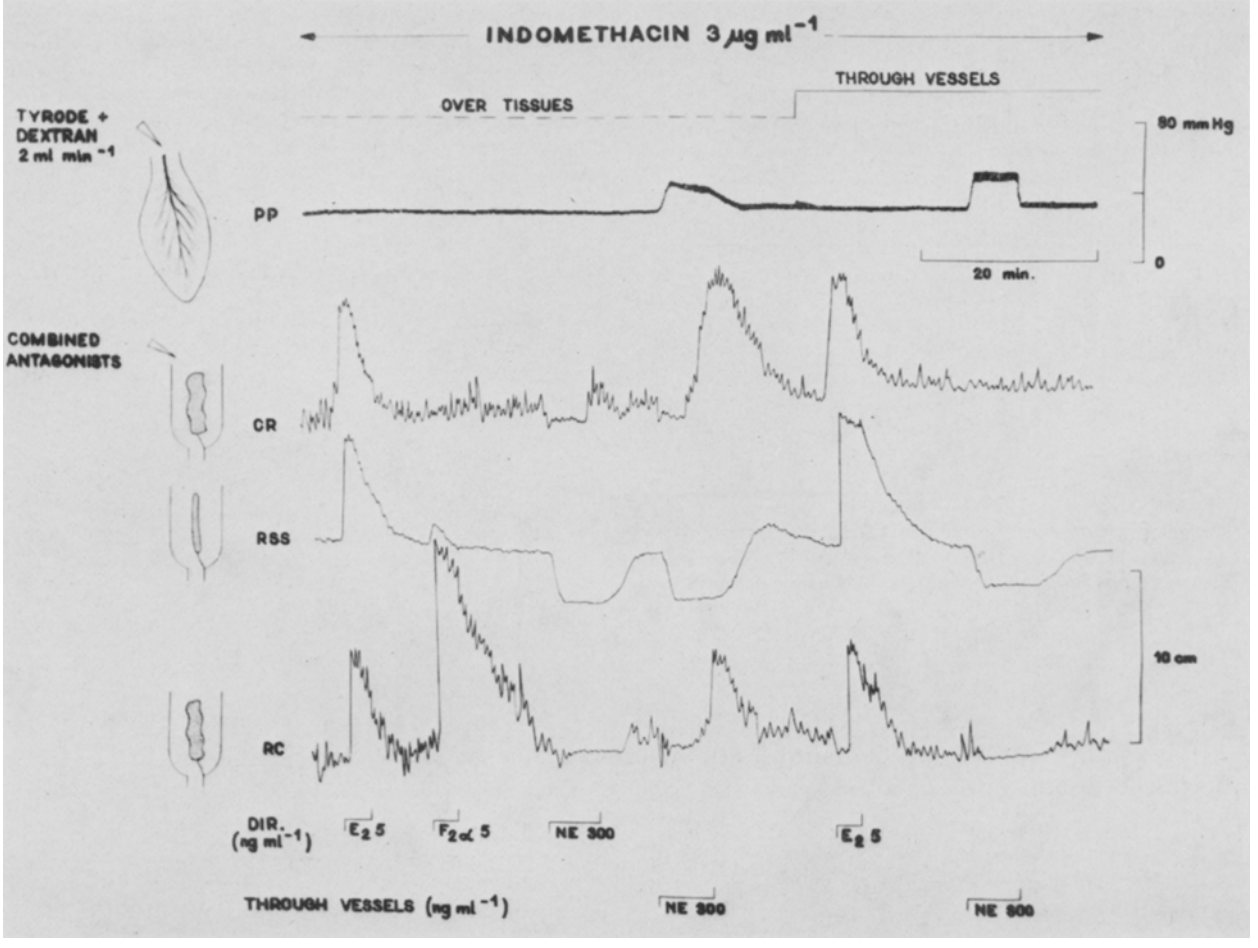

Release of PGE from perfused rabbit ear by norepinephrine (NE, 300 ng/ml) and its blockade by indomethacin (3 μg/ml). The assay organs: the chick rectum (CR), the rat stomach strip (RSS) and the rat colon (RC) were superfused in cascade with the effluent from the ear vessels. The sensitivity of the assay organs was calibrated (DIR). PP is the perfusion pressure. The peak concentration of a substance released by NE was 6.2 ng/ml in terms of PGE_2-like activity. Scales: time 20 min, contraction of the assay organs 10 cm and PP 80 mm Hg.

effect. The ratio of an initial increase in perfusion pressure to the remaining increase measured at the end of the infusion period was considered as an index of acute tolerance to NE. In 11 experiments the percent of the increase in initial perfusion pressure was 67 ± 9%, and the index of acute tolerance was 1.54 ± 0.16 (mean ± SEM). At the time of an infusion, there appeared in the effluent a substance contracting the chick rectum and rat colon (Figure). On the basis of differential sensitivity of both organs to different types of PG, it could be suspected that there was released a PGE-like material. Its peak concentration in the effluent in terms of PGE_2-like activity was 5.08 ± 0.72 ng/ml (9 experiments). If a PGF-like substance was released into the effluent, then its concentration had to be lower than 0.5 ng/ml. Infusions of NE at a concentration higher than 300 ng/ml sometimes produced an appearance of PGF-like material in the effluent, but then no acute tolerance of pressor response was observed.

The prostaglandin character of the released substance was confirmed by the pretreatment of the ear vessels with an inhibitor of PG biosynthesis-indomethacin (3 µg/ml). In the presence of indomethacin NE did not release a substance contracting the assay organs (8 experiments, Figure), but an initial increase in perfusion pressure was the same as in control experiments (69 ± 8%), while the index of acute tolerance was significantly lower (1.05 ± 0.04, $p < 0.01$) than in control experiments. The pretreatment of the ear vessels with phenoxybenzamine, but not with propranolol (6 experiments) completely blocked the pressor response to NE, and then there was no release of PGE-like substance into the effluent.

Therefore we assume that contracting vascular wall produces PGE, which are responsible for acute tolerance to NE. Many authors[9,13,14] have demonstrated that exogenous PGE diminish the vasoconstrictor action of NE. There is also indirect evidence that the intramural generation of PGE in blood vessels may be a feedback mechanism limitting vasoconstrictor action of catecholamines[9,15] or angiotensin[10]. Our data support this concept.

Zusammenfassung. Durch Noradrenalin ausgelöste Vasokonstriktion am perfundierten Kaninchenohr ist begleitet von der Freisetzung eines PGE-ähnlichen Stoffs, welcher für die Entwicklung der akuten Toleranz gegenüber der vasokonstriktorischen Wirkung von Noradrenalin verantwortlich ist.

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Stimulation of Sexual Behaviour in Rats by a Benzodioxane Derivative

In the course of screening potential centrally acting muscle relaxants, it was noticed by Morrison[1] that young adult male Wistar rats which were housed in groups of 10 showed unusually excited sexual behaviour some hours after the administration of a benzodioxane derivative, 2-(3′*tert*-amylsulphonylpropyl)aminoethyl-1:4-benzodioxane (WB 4371) (Figure 1). Effects of this kind have previously been shown mainly with hormones and with *p*-chlorophenylalanine (pCpA), and recently also with L-dopa[2]. Benzodioxanes have hitherto been regarded as predominantly hypotensive and/or CNS depressant.

Pilot experiments were also carried out with a number of other benzodioxane derivatives, but WB 4371 was found to be the most potent of the series in stimulating sexual activity. Experiments were therefore done to quantify and analyze these effects, and also to compare WB 4371 with pCpA which has been reported, for example, to enhance sexual behaviour in male rats[3–7] and in cats of both sexes[6–9], though opposite effects have also been shown[10].

All experiments were carried out with naive adult male Wistar rats (200–250 g) which had been housed in standard conditions in large cages containing 12 rats each. All injections were i.p. and WB 4371 was dissolved and pCpA was suspended in 1% Tween 80. Observations of sexual activity were made 4 to 5 h after injection with WB 4371, so as to allow its initial CNS depressant effects to wear off, and 6 h after injection with pCpA, to allow its sedative effects to wear off. Rats were tested in an observation cage in groups of 4, each animal having received the same treatment. The sexual acts scored were similar to those described by Grant and McIntosh[11]. All tests lasted 15 min and took place in the afternoon. For practical reasons the rats were not kept in a reversed day/night light cycle.

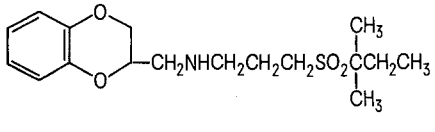

Fig. 1. Chemical structure of benzodioxane derivative WB 4371.

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