tron spin resonance technic[19]. Unfortunately, Gbe interferes with color development in the first method and the freezing technic used in the second method gives unreproducible results so that it has not been proved in an indisputable way that Gbe scavenges O_2^-.

3) Gbe strongly scavenges $OH^\cdot$ generation in these experiments on PMNs. It is efficient at concentration as low as 15.6 µg/ml, while no effect is observed on O_2 uptake or on O_2^- and H_2O_2 release at this concentration.

This can be explained by the powerful free radical scavenging activity of Gbe, previously described in in vivo and in vitro experiments[20-22]. It easily reacts not only with $OH^\cdot$ but also with the 2,2-diphenylpicrylhydrazyl radical (DPPH) and the adriamycyl radical. Gbe also reduces in this way the free radical-induced lipoperoxidation generated by the NADPH-Fe^{3+} system in rat microsomes[9].

4) The myeloperoxidase activity which is responsible for the generation of strong oxidant species (HOCl, chloramines) is also significantly reduced by Gbe, even at low concentration (IC_{50} = 10 µg/ml).

Because of its regulator action on PMNs functions (inhibition of the NADPH-oxidase activity with, as a consequence, a decrease of release of activated oxygen species; scavenging of the hydroxyl radical, an oxidant species inducing lipoperoxidation phenomena; inhibition of myeloperoxidase activity), Gbe appears to be an interesting therapeutic agent. Indeed, our results give support to recent studies which have shown that PMA-induced superoxide anion release in polymorphonuclear cells from whole body gamma irradiated rabbits was significantly reduced in animals treated preventively with Gbe[23]. Besides its radiobiological protection, Gbe has been suggested for the reduction of the post radiotherapic edema in larynx cancer treatment, where there is an increase of O_2^- release by PMNs[24]. On the other hand, Gbe could be used experimentally in the treatment of diseases where free radical production by PMNs is suspected, as is the case in the Adult Respiratory Distress Syndrome (ARDS)[25,26] or in burn-injured patients[27].

Conclusion. Ginkgo biloba extract significantly reduces the production of oxygen species (O_2^-, H_2O_2 and $OH^\cdot$) during stimulation of human neutrophils by phorbol myristate acetate. Further studies are required to show the action of the extract on the chlorine derivatives mediated by myeloperoxidase and, on the other hand, to determine which fraction in the extract is the most active.

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Tetanus toxin does not affect the release of noradrenaline and taurine from rat cerebral cortex slices evoked by high K^+ and Na^+-free media

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Summary. Noradrenaline and taurine release from superfused rat cerebral cortex slices was stimulated by potassium ions, veratrine, ouabain and omission of sodium ions. Tetanus toxin enhanced only the ouabain-evoked calcium-dependent noradrenaline release and the ouabain-evoked calcium-independent taurine release. The uptake of both was marginally affected.
Key words. Brain slices; noradrenaline; taurine; evoked release; uptake; tetanus toxin.

Tetanus toxin interferes with the release of several neurotransmitters from nerve endings[1]. Its main targets are the spinal inhibitory interneurons and their afferent terminals[2], in which the release of glycine and GABA is blocked. Consequently, the spinal motoneurons become hyperactive, giving rise to the spasticity and rigidity characteristic of manifest tetanus. In studies in vitro the type of tissue preparation used and the nature of the stimulus used to elicit the transmitter release are of impor-

Table 1. Influx and accumulation of [^{3}H] noradrenaline in rat cerebral cortex slices in the presence of tetanus toxin

Treatment of slices	Concentration ratios when [^{3}H] noradrenaline present during		
	First 10 min	Last 10 min	All 120 min
Controls	0.79 ± 0.02 (10)	0.99 ± 0.02 (8)	4.12 ± 0.08 (23)
Toxin-treated	0.91 ± 0.03 (10)*	1.03 ± 0.02 (9)	4.58 ± 0.11 (21)**

Mean ± SEM with the number of experiments on different rats in brackets. Significance of differences (Student's independent t-test): *$p < 0.05$; ** $p < 0.01$.

Table 2. Influx and accumulation of [^{3}H] taurine in rat cerebral cortex slices in the presence of tetanus toxin

Treatment of slices	Concentration ratios when [^{3}H] taurine present during		
	First 10 min	Last 10 min	All 120 min
Controls	1.87 ± 0.15 (4)	3.20 ± 0.15 (4)	35.97 ± 1.42 (5)
Toxin-treated	1.79 ± 0.06 (3)	2.31 ± 0.11 (4)*	34.72 ± 2.44 (4)

Mean ± SEM with the number of experiments on different rats in brackets. Significance of differences: *$p > 0.001$.

tance. For instance, in a previous work of ours tetanus toxin attenuated the GABA release evoked by an excess of potassium ions or by the omission of sodium ions, but not the release induced by veratrine and ouabain[3]. Although the release of glycine and GABA, the two main inhibitory transmitters in the mammalian nervous system, is often affected by the toxin, its influence on the release of other neurotransmitters is less consistent.

Noradrenaline is a transmitter liberated by exocytosis of synaptic vesicles[4], while the releasable GABA apparently originates preferentially in the cytosol[5]. This important difference prompted us to study in vitro the effects of tetanus toxin on noradrenaline release elicited by the above-mentioned stimuli, in order to see whether or not the tetanus-toxin action depends on the storage site of the transmitter. Taurine is an inhibitory amino acid like GABA and glycine, though it has not been conclusively shown to be a neurotransmitter[6,7]. It is, however, enriched by synaptic vesicles[8] and released from nerve endings by depolarizing stimuli[9]. The effects of tetanus toxin on taurine uptake and release were also assessed here, since the possible effects of the toxin on the synaptic actions of taurine are at present entirely unknown.

Cerebral cortex slices (0.5 mm thick, 15–25 mg) were prepared from male Sprague-Dawley rats (150-250 g) using a Stadie-Riggs tissue slicer. The incubation and superfusion medium for the slices was Krebs-Ringer-Hepes solution of the following composition (in mM): NaCl 127, $MgSO_4$ 1.3, KCl 5, $CaCl_2$ 0.75, Hepes 15, NaOH 11, and D-glucose 10. pH was adjusted to 7.4 at 310 K. The toxin concentration in all experiments was 1.5×10^6 MLD/l (minimal lethal doses per liter). The concentrations of noradrenaline and taurine in the medium were 1 µM (40 Bq/l), and 10 µM, (20 MBq/l), respectively. In the experiments with noradrenaline the medium also contained 2 µM tranylcypromine to prevent noradrenaline metabolism. In Na^+-free medium NaCl was equimolarly replaced by choline chloride, and in medium which contained 50 mM K^+ an equivalent amount of Na^+ was omitted. Ca^{2+}-free media were supplemented with 0.5 mM EGTA (ethyleneglycol-bis-(β-aminoethylether)-N, N'-tetraacetic acid).

In some experiments designed for estimation of initial influx rates the incubation time with the toxin and labeled compounds was 10 min. In all other experiments the incubation period was 2 h, i.e. the minimum time required by the toxin to become internalized in the cells and to have an effect[3]. After incubation the

Table 3. Effect of tetanus toxin in [^{3}H]noradrenaline release from rat cerebral cortex slices induced by K^+ ions, veratrine, ouabain and by an omission of Na^+ions, all with and without calcium

Treatment of slices	Efflux rate constants ($\times 10^{-3}$ min^{-1})	
	Basal	Stimulated
		50 mM K^+
Controls (9)	17.48 ± 0.82	28.20 ± 1.68
Toxin (10)	18.77 ± 0.36	30.63 ± 0.91
		50 mM K^+, Ca^{2+}-free
Controls (4)	14.16 ± 0.42	13.85 ± 0.48
Toxin (3)	15.04 ± 0.41	14.44 ± 0.68
		25 µM veratrine
Controls (5)	16.66 ± 0.28	31.26 ± 1.76
Toxin (5)	17.13 ± 1.27	35.29 ± 2.27
		25 µM veratrine, Ca^{2+}-free
Controls (8)	13.63 ± 0.83	17.34 ± 1.17
Toxin (9)	12.82 ± 0.68	15.29 ± 0.91
		Na^+-free
Controls (7)	14.02 ± 0.48	23.90 ± 0.71
Toxin (8)	12.92 ± 1.19	23.96 ± 1.26
		Na^+-free, Ca^{2+}-free
Controls (5)	10.68 ± 0.55	10.63 ± 0.84
Toxin (4)	11.68 ± 0.82	11.15 ± 0.82
		200 µM ouabain
Controls (4)	16.92 ± 1.60	28.19 ± 0.63
Toxin (5)	15.85 ± 0.75	39.30 ± 3.26*
		200 µM ouabain, Ca^{2+}-free
Controls (4)	12.58 ± 0.49	14.43 ± 0.67
Toxin (4)	13.72 ± 1.11	13.93 ± 0.68

Mean ± SEM with the number of experiments on different rats in brackets. Significance of differences from the corresponding control values: *$p < 0.05$.

Table 4. Effect of tetanus toxin in [^{3}H]taurine release from rat cerebral cortex slices induced by K^+ ions, veratrine, ouabain and by an omission of Na^+ions, all with and without calcium

Treatment of slices	Efflux rate constants ($\times 10^{-3}$ min^{-1})	
	Basal	Stimulated
		50 mM K^+
Controls (3)	3.15 ± 0.40	8.63 ± 0.25
Toxin (4)	2.97 ± 0.31	8.43 ± 0.37
		50 mM K^+, Ca^{2+}-free
Controls (4)	4.86 ± 0.30	7.41 ± 0.47
Toxin (3)	4.46 ± 0.33	7.89 ± 0.68
		25 µM veratrine
Controls (4)	3.00 ± 0.24	8.53 ± 0.37
Toxin (3)	2.51 ± 0.14	7.64 ± 0.69
		25 µM veratrine, Ca^{2+}-free
Controls (4)	5.48 ± 0.23	8.20 ± 0.06
Toxin (4)	5.96 ± 0.59	8.67 ± 0.44
		Na^+-free
Controls (5)	3.43 ± 0.49	32.49 ± 0.72
Toxin (4)	1.94 ± 0.33	29.94 ± 3.85
		Na^+-free, Ca^{2+}-free
Controls (4)	7.22 ± 0.77	20.22 ± 1.96
Toxin (5)	5.99 ± 0.41	22.23 ± 1.06
		20 µM ouabain
Controls (4)	2.25 ± 0.11	4.24 ± 0.67
Toxin (4)	3.09 ± 0.48	4.84 ± 0.60
		20 µM ouabain, Ca^{2+}-free
Controls (5)	6.69 ± 0.57	12.39 ± 1.24
Toxin (4)	6.27 ± 0.23	17.53 ± 1.70*

Mean ± SEM with the number of experiments on different rats in brackets. Significance of differences from the corresponding control values: *$p < 0.05$.

slices were rinsed with fresh medium and measured for radioactivity or transferred to small (250 µl) agitated glass cups, one slice into each, to be superfused with Krebs-Ringer-Hepes medium at a rate of 0.25 ml/min. Both incubation and superfusion were carried out under pure oxygen at 310 K in a shaking water bath. The superfusions were commenced with plain Krebs-Ringer-Hepes medium up to the first 30 min. The period from 20 to 30 min served as period for 'basal' efflux of each individual slice. Stimuli with modified media were applied for 10 min from 30 to 40 min. The superfusates were collected in 2-min (0.5 ml) fractions for radioactivity determination. After the superfusions the slices were weighed, homogenized in 5% (w/v) trichloracetic acid solution and centrifuged, and the resulting clear supernatants measured for radioactivity by liquid scintillation counting. The efflux rate constants for various experimental phases were computed as negative slopes for the regression lines of the logarithm of radioactivity percentage remaining in the slices versus superfusion time[10].

Neither the uptake of noradrenaline nor that of taurine was markedly affected by tetanus toxin. The initial noradrenaline uptake increased slightly when tetanus toxin was present extracellularly but not after the internalization of the toxin, i.e. during the last 10 min of incubation (table 1). In contrast, taurine uptake was only then reduced by the toxin (table 2). Both taurine[11] and the toxin[12] are subject to vinblastine-sensitive fast axonal transport and thus the toxin may interfere with taurine transport at the level of the microtubular system of nerve endings. The long-term accumulation of noradrenaline was influenced by the toxin, nor was that of taurine.

Noradrenaline, a catecholamine, is stored in synaptic vesicles in the brain with a tissue concentration in the micromolar range[13], while taurine, an amino acid, is present at millimolar concentrations with only a fraction sequestered to synaptic vesicles[8]. In spite of these differences, the effects of tetanus toxin on the release of both compounds elicited by various chemical stimuli exhibited a similar pattern; only the ouabain-evoked release was enhanced by the toxin (tables 3 and 4). The responses to the two other stimuli, K^+ ions and veratrine, which depolarize the cells and induce influx of Na^+ ions, and the response to the omission of sodium ions, were not modified by tetanus toxin.

The evoked release of noradrenaline was strictly dependent on Ca^{2+} ions with all types of stimuli (table 3). The basal efflux of taurine was increased by the absence of Ca^{2+} ions from the superfusion medium (table 4). However, the evoked release of taurine was only slightly influenced by the omission of Ca^{2+}, except in the case of ouabain stimulation. Then tetanus toxin also caused an enhancement which was of about the same magnitude as with noradrenaline. The effect of the toxin on the ouabain-evoked release thus manifests itself only when in the presence (noradrenaline) or in the absence (taurine) of calcium the efflux was significantly increased over the basal levels. The concentrations of ouabain required to elicit a stimulation had to be 10 times as great with noradrenaline as the concentration effective with taurine.

Our previous studies have shown that a slight enhancement similar to the above also occurs in the ouabain-evoked GABA release[3]. The intracellular presence of tetanus toxin thus seems to cause a general increase in neurotransmitter release when Na^+, K^+-ATPase is inhibited by ouabain. The gangliosides G_{M1}, G_{D1b} and G_{T1b} increase the activity of Na^+, K^+-ATPase[14] and tetanus toxin has a high affinity for the two last-mentioned gangliosides[15]. However, we prefer not to conclude that there is a direct interaction with the toxin and Na^+, K^+-ATPase, since there is no apparent reason why the basal unstimulated release of neurotransmitters would not also be enhanced by the toxin, and this was nevertheless not the case.

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Central neural control of pineal melatonin synthesis in the rat[1]

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Summary. To investigate a possible central neural influence on nocturnal pineal metabolic activity in rats, frontal transsections of the stria medullaris thalami were conducted. Enzymes involved in melatonin synthesis, i.e. N-acetyltransferase and hydroxyindole-O-methyl-transferase, exhibited reduced activities in operated animals when compared to controls. These results indicate a modulatory role of central structures on nocturnal pineal indole metabolism.
Key words. Central innervation; melatonin synthesis; pineal gland; rat.