The drugs used were: L-noradrenaline bitartarate (Serva), phentolamine HCl (Ciba-Geigy) and D,L-propanolol (I.C.I.), the doses are expressed in the term of the salts. Student t-test was used for evaluation.

Results. Sham injections and physiological saline had no significant effect on colonic temperature. Propranolol i.p. or phentolamine i.p. alone induced a fall in Tc and at the moment of NA injection Tc-s were lower than initially. 1 mg/kg propranolol blocked the effect of 100 µg/kg NA i.m., whereas the effect of 10 µg NA injected i.c.v. was unaffected. 30 µg propranolol injected into the lateral cerebral ventricle had no effect on the action of systemically administered NA and did not decrease the effect of NA injected into the ipsilateral or contralateral cerebral ventricle (figure 1). Practolol had the same effect as propranolol. 1 mg/kg phentolamine i.p. did not alter the effect of 100 µg/kg NA i.m., whereas it decreased the effect of 10 µg NA i.c.v., 30 µg phentolamine i.c.v. had no effect on the action of systemically administered NA, whereas the effect of 10 µg NA i.c.v. was blocked completely (figure 2). Phenoxybenzamine and ergotamine acted as phentolamine.

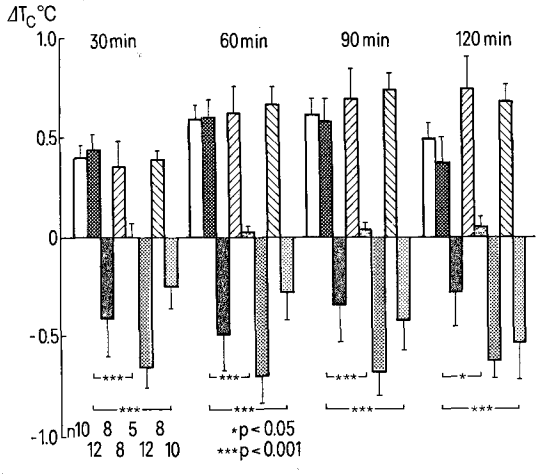

Fig. 2. The effect of 100 µg/kg NA i.m. (□), 10 µg NA i.c.v. (■), 1 mg/kg phentolamine i.p. (▩), 1 mg/kg phentolamine i.p. + 100 µg/kg NA i.m. (▨), 1 mg/kg Phentolamine i.p. + 10 µg NA i.c.v. (▩), 30 µg phentolamine i.c.v. (▩), 30 µg phentolamine i.c.v. + 100 µg/kg NA i.m. (▨) and 30 µg phentolamine i.c.v. + 10 µg NA. i.c.v. (▩) on colonic temperature 30, 60, 90 and 120 min after NA.

Discussion. NA elicited a rise in Tc in the newborn guinea-pig, independently of the route of administration. The mechanism of action was, however, not identical.

Beta-adrenergic blockers i.p. blocked the effect of systemically administered NA, implying the effect of NA was mediated by peripheral beta-adrenergic receptors. The effect of centrally applied NA was, however, not inhibited by i.p. administered beta-blockers, and beta-blockers injected into the lateral cerebral ventricle even enhanced the effect of NA. This observation indicates that, in contrast to systemically administered NA, the effect of centrally applied NA on Tc is not being mediated by beta-adrenergic receptors.

Alpha-adrenergic receptor blockers administered i.p. or i.v. failed to block the effect of systemically administered NA. In contrast, the effect of NA injected into the lateral cerebral ventricle was diminished by i.p. injected, and blocked by i.c.v. administered alpha-adrenergic blockers, indicating that the effect of centrally applied NA is mediated by central alpha-adrenergic receptors.

Despite the fact that the magnitude of the response to NA decreased with age, the effects of both alpha- and beta-blockers were not subjected to change with age up to the age of 12 days.

In vitro mutagenicity of the soil nematicide 1,3-dichloropropene

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Summary. The cis- and trans-isomers of 1,3-dichloropropene have been tested in the Ames mutagenicity assay system on Salmonella typhimurium tester strain TA 1535. Both isomers have been found to be mutagenic even without microsomal activation.

Some chlorinated olefines have recently been identified as carcinogens and/or mutagens, like vinyl chloride in experimental animals[1-3] and man[4], vinylidene chloride and 2-chlorobutadiene in in vitro mutagenicity testing[5,6], trichloroethylene in intact animal carcinogenicity testing[7] and in in vitro mutagenicity test[8]. The hypothesis has been promoted that all these compounds are activated in mammalian metabolism to electrophilic epoxides which may easily react, by alkylation, with essential cellular macromolecules[9,10]. A similar mechanism was suspected to prevail with 1,3-dichloropropene (1,3-DCP), a compound used as soil nematicide in agriculture[11]. To test this hypothesis, we used the Ames mutagenicity assay system[12].

The technical product of 1,3-DCP usually consists of a mixture of the cis- and trans-isomers with a small amount of 3.3-dichloropropene as an impurity. We have tested both the cis- and trans-isomers of 1,3-DCP. Purity according to GC-analysis: cis-1,3-DCP 99.97% (contaminants: 0.01% 3,3-dichloropropene-1, 0.02% 1,2-dichloropropane); trans-1,3-DCP 97.46% (contaminants: 1.32% 3,3-dichloropropene-1, 0.37% cis-1,3-DCP, 0.85% 'heavy ends'). Both isomers have been found to be mutagenic (table). Salmonella thyphimurium tester strains TA 1535, TA 1537 and TA 1538 all gave positive results. The data presented in this communication were obtained with TA 1535 which can detect mutagens causing base pair substitution.

The table shows the typical result of 1 out of several assays we made to prove the mutagenicity of 1,3-DCP. All assays gave essentially similar results: Both isomers of 1,3-DCP are mutagenic, even without microsomal activation, the cis-isomer being more active than the trans-isomer by the factor of about 2. There is also a significant difference in the survival rate of the bacteria exposed to varying concentrations of both isomers. At all concentrations tested, survival rates of cells exposed to cis-DCP are generally lower than those of bacteria exposed to trans-DCP. Concentrations of 2 µl/ml or more cause a drop of the survival rate to less than 1%, both in the case of cis- and trans-DCP.

Results of a typical experiment of mutagenicity testing of cis- and trans-isomers of 1,3-dichloropropene (DCP) with Salmonella thyphimurium TA 1535, with and without S9

Mutagen	Concentration (µl/ml top agar)	Survivors ($\times 10^8$) Mean value of 3 plates	%Survival	Revertant colonies per plate Mean value of 3 plates ± SE
cis-DCP	S9 Absent			
	–	0.96	100	15 ± 2.64
	0.1	0.70	72.9	215 ± 10.59
	0.5	0.38	39.6	456 ± 15.55
	1.0	0.04	4.2	146 ± 29.68
	S9 Added			
	–	0.81	100	11 ± 1.73
	0.1	0.81	100	72 ± 3.71
	0.5	0.62	76.5	287 ± 2.02
	1.0	0.29	35.8	434 ± 21.2
trans-DCP	S9 Absent			
	–	0.96	100	15 ± 2.64
	0.1	0.92	95.8	110 ± 8.14
	0.5	0.64	66.7	288 ± 8.02
	1.0	0.22	22.9	359 ± 7.12
	S9 Added			
	–	0.81	100	11 ± 1.73
	0.1	0.87	107.4	32 ± 4.58
	0.5	0.78	96.3	110 ± 6.11
	1.0	0.49	60.5	217 ± 2.08

The bacteria were grown in nutrient broth, shaken for 12 h at 37 °C, and 0.1 ml was then added to the molten top agar, with and without 0.5 ml of 'S-9-mix'[12]. This mix contained per ml: 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP, 100 mM sodium phosphate (pH 7.4) and 0.3 ml of liver homogenates (S-9) (9000 × g supernatant) from male Wistar rats (of about 250 g each) which were induced by a single i.p. injection of a polychlorinated biphenyl (PCB) mixture (Aroclor 1254), diluted in corn oil to a concentration of 200 mg/ml. A dosage of 500 mg/kg was given to each rat 5 days before sacrifice. DCP was diluted 100fold in dimethylsulfoxide (DMSO) and equivalent volumes to those listed in the table were added directly to the top agar. Triplicate petri plates containing Vogel-Bonner E medium[13] were overlayed with this mixture and incubated at 37 °C. After 48 h the revertant colonies were counted. For determination of survival rates, the top agar mixture was poured on triplicate petri plates containing Vogel-Bonner E medium with 8% nutrient broth. In this case, the bacteria have been diluted by the factor of 10^8 in 0.9% NaCl before addition to the top agar. The colonies of surviving bacteria were counted after 24 h of incubation at 37 °C.

Surprizingly there is not only no enhancement but even a marked reduction of the rate of back mutations after addition of microsomes. Moreover, the cytotoxicity of both isomers is also drastically reduced. The reason for this unexpected finding still has to be revealed. To make sure that the S9-mix used was enzymatically active, a control series of 6 plates with Vogel-Bonner E medium was overlayed with top agar containing 40 µg of 2-aminoanthracene (in 100 µl of DMSO), with and without S9-mix, and treated in the same way as the other plates. 721 ± 9.36 colonies of revertants were counted on the (triplicate) plates with S9-mix, 11 ± 2.21 colonies on those without it. We anticipate a direct alkylating reactivity of DCP because, according to preliminary experiments, both isomers of DCP rapidly react with 4-nitrobenzpyridine.

The data shown strongly suggest further investigation on 1,3-DCP as a potential carcinogen and demonstrate that it is a potent mutagen under the experimental conditions as described.

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Intracellular localization of calcitonin in the C cells of the rat[1]

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Summary. Specific localization of C cells at the electron microscope level was achieved by an indirect immunoperoxidase technique. The hormone is present in the electron dense granules. The presence of granules apparently devoid of calcitonin was also detected.

Specific localization of calcitonin is a perequisite for the study of the secretion of the hormone at the ultrastructural level. We have shown that antibodies, raised in the the rat, directed towards human calcitonin can be used for the specific localization of calcitonin producing cells (C cells) in the rat using a double immunofluorescence technique[3]. In the present work, we have studied the ultrastructural localization of calcitonin in the rat C cells, using a double immunoperoxidase technique.

Material and methods. 1-mm³ blocks of thyroid glands of male Wistar rats, 100 g b.wt were fixed for 1 h at 4°C in 2.5% glutaraldehyde in phosphate buffer 0.1 M pH 7.2,

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