Smallest zinc quantities affect the histamine release from peritoneal mast cells of the rat

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Summary. Seven individual 0.025-mg doses of zinc administered as lactose tablets on consecutive days, significantly increase histamine release from peritoneal mast cells of the rat. Seven individual doses of 0.25 µg cause a somewhat smaller, though still very pronounced increase in the release in comparison with zero control.
Key words. Zinc; histamine release; peritoneal mast cells.

The effect of zinc ions on histamine release from the peritoneal mast cells of the rat has been well investigated recently[1-3]. The origin of the zinc effect is mostly referred to an effect of zinc-dependent peptidases directly or indirectly participating in the release process[4,5]. The most important results of these investigations are that the effect of zinc for in vitro doses is not linear and that very small amounts are still effective.

Material and methods. The mast cell model must be suitable for detection of any possible effect of homoeopathically prepared zinc dilutions and – in the positive case, at least for zinc – be able to prove the scientific demonstrability of homoeopathy.

To investigate this aspect, a commercially available lactose tablet (Fa. Deutsche Homöopathie-Union, Karlsruhe, FRG) was orally administered to individually caged male Wistar rats (250 ± 10 g b.wt) on seven consecutive days at the same time of day (0.900 h). Depending on experimental group, these tablets contained 0.025-mg (D4), 0.25-µg (D6) and 0.25-pg (D12) zinc. D4 stands for the 4th decimal potency corresponding to 1 g zinc contained in 10^4 g lactose, D6 = the 6th decimal potency (1 g zinc in 10^6 g lactose), and D12 = the 12th decimal potency (1 g zinc in 10^{12} g lactose). One group received lactose tablets for reference purposes (placebo), and one group remained untreated as a zero control.

24 h after the last individual administration, peritoneal mast cells were obtained from ether-anesthetized animals by peritoneal lavage with 20 ml PIPES-Glucose-Heparin buffer (25 mM PIPES, 0.4 mM $MgCl_2$, 5 mM KCl, 5.6 mM glucose, 1 mM $CaCl_2$, 200 U/ml Heparin-Na, pH 7.0; 277.1 °K)[3,6] and then separated from other peritoneal cells by 22.5% (w/v) metrizamide (centrifugation 20 min, 180 × g, 277.1 °K)[7,8]. Some 7.5×10^5 non-stimulated mast cells were thus obtained per rat. This is approximately 10% of the total cell count of the peritoneal lavage fluid. The mast cells obtained in this manner were resuspended in 0.6 ml PIPES-buffer without glucose and heparin[3], divided into aliquot parts, and incubated at 310.1 °K. Under these conditions, mast cells release some amount of the histamine contained in them. The indication of released histamine is given as a percentage of the total histamine[3,9]. This is composed of the released histamine and the histamine retained in the mast cells (fig. 1) and was measured fluorometrically[10,11].

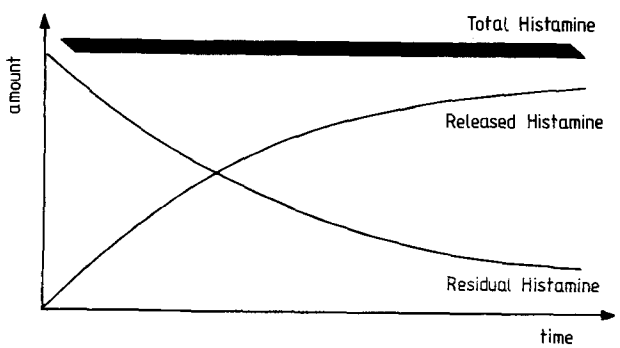

Figure 1. Characteristic of the histamine release from rat peritoneal mast cells after incubation.

Results and discussion. Pretreatment of the rat with zinc influences the quantity of the histamine released under incubation conditions and the release characteristics. The administration of seven individual 0.025-mg doses, i.e. a total dose of 0.175-mg zinc per rat, causes a major rise in the histamine release in comparison with the zero control. Seven individual 0.25-µg doses, corresponding to a total dose of 1.75-µg, also cause a similarly pronounced increase in the release. In the case of administration of seven individual 0.25-pg doses, the rise in the release is still only detectable for incubation periods of 20 and 30 min (fig. 2).

On the basis of the results presented here, it must be said that the histamine release from peritoneal mast cells is influenced already at very small zinc quantities. Such small quantities are not generally used. On these grounds, scarcely any data are available for this dose range[12]. The fact that the zinc used here was produced according to the rules for the preparation of homoeopathic medications, i.e. in consecutive 1 to 10 dilution-steps with respective intensive trituration, has no fundamental significance. The material quantities in the milligram and microgram range are fully able to exert functional effects on mast cell metabolism. The effect range of the smallest doses, for which an example elaborated according

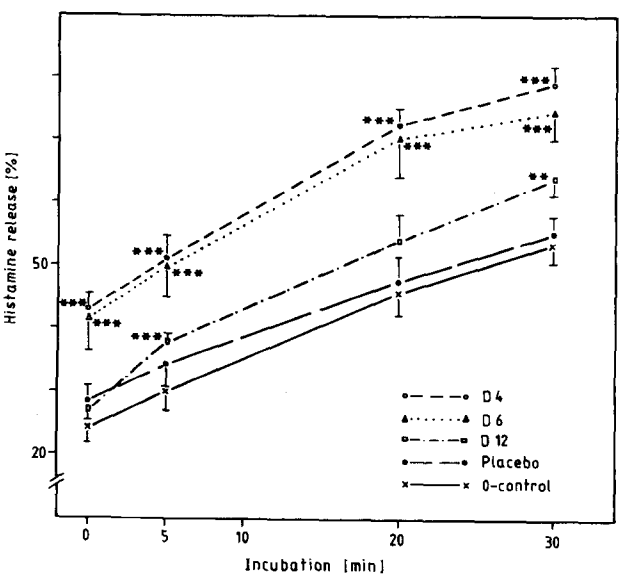

Figure 2. Histamine release from rat peritoneal mast cells after pretreatment with seven individual doses of zinc administered orally at 09.00 h (concentrations see text); aliquots of 1.5×10^5 mast cells were incubated for 0, 5, 20, or 30 min at 310.1 °K; each group consisted of 6 individually caged animals (250 ± 10 g b.wt); statistic: vertical bars represent SD, comparison of the zinc values with corresponding 0-control (untreated animals), t-Test: ** = $p < 0.01$; *** = $p < 0.001$.

to rigorous scientific criteria has been given here, is of such profound general importance as to merit further detailed investigation. Speculation as to the origin of such effects should be avoided.

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1 Kazimierczak, W., and Maslinski, C., Agents Actions *4* (1974) 1.
2 Struckhoff, T., and Heymann, E., Biochem. J. *236* (1986) 215.
3 Harisch, G., and Kretschmer, M., Res. Commun. Chem. Path. Pharmac. *55* (1987) 39.
4 McDonald, J. K., Callahan, P., Smith, R. E., and Ellis, S., in: Tissue Proteleinases, p. 69. Eds A. J. Barret and J. T. Dingle. Amsterdam 1971.
5 McDonald, J. K., and Schwabe, C., in: Proteinases in Mammalian Cells and Tissues, p. 311. Ed. A. J. Barret. Amsterdam 1977.
6 White, J. R., and Pearce, F. L., Immunology *46* (1982) 361.
7 Coutts, S. M., Nehring, R. E., and Jariwala, N. U., J. Immun. *124* (1980) 2309.
8 Yurt, R. W., Leid, W., Spragg, J., and Austen, K. F., J. Immun. *118* (1977) 1201.
9 Heiman, A. S., and Crews, F. T., J. Immun. *134* (1985) 548.
10 Anton, A. M., and Sayre, D. F., J. Pharmac. exp. Ther. *166* (1969) 285.
11 Siriganian, R. P., Analyt. Biochem. *57* (1974) 383.
12 Davenas, E., Poitevin, B., and Benveniste, J., Eur. J. Pharmac. *135* (1987) 313.

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Enantiomeric cannabinoids: stereospecificity of psychotropic activity

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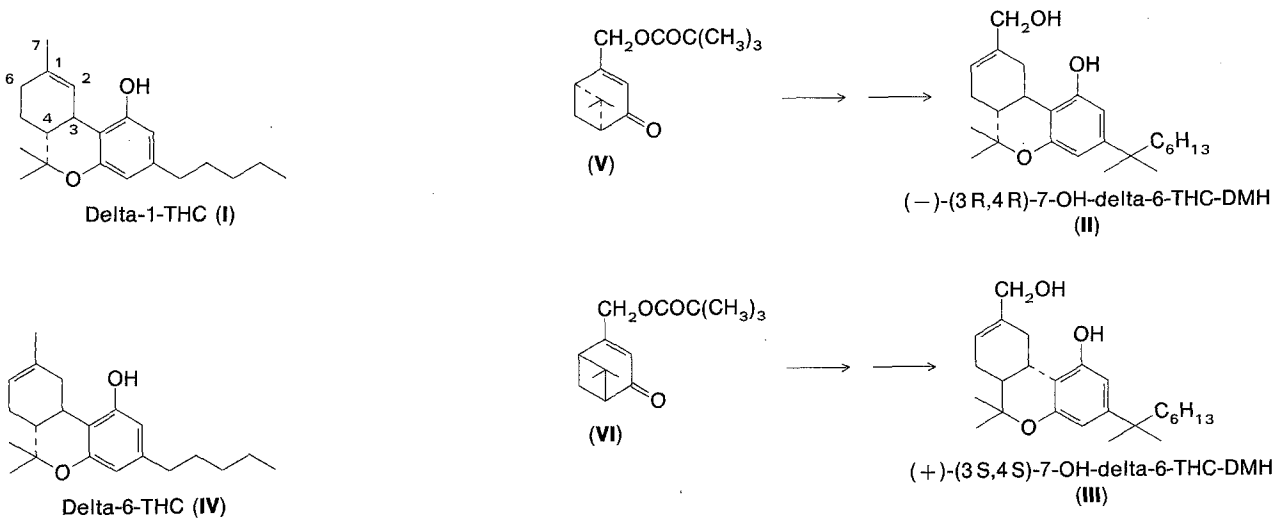
Summary. The 1,1-dimethylheptyl homolog of (−)-(3R,4R)-7-hydroxy-delta-6-tetrahydrocannabinol (compound **II**) is highly psychotropic in mice, rats and pigeons. The (+)-(3S,4S) enantiomer (**III**) was found to be psychotropically inactive at doses up to several thousand times those of the ED_{50} of (**II**).
Key words. Cannabinoids; stereospecificity; psychotropic activity; drug discrimination.

The stereochemical requirements for cannabimimetic activity are not considered to be very high[3]. Cannabinoids with (3R,4R) configuration, which is the stereochemistry of natural delta-1-tetrahydrocannabinol (delta-1-THC) (**I**), are reported to be only about 10–20 times more active in tests for psychotropic activity than the corresponding synthetic (3S,4S) enantiomers. This presumed relatively low stereospecificity may have biological implications. Indeed, in a recent review[4] it was pointed out that 'while cannabinoid SAR supports the concept of a specific cannabinoid receptor, a disconcerting element is the apparent lack of greater stereoselectivity in some animal models.' However, most tests with synthetic (3S,4S) cannabinoids reported so far seem to have been performed with compounds of doubtful enantiomeric purity. Most, if not all (3S,4S) THC derivatives tested so far have been prepared following a route put forward by our group nearly 20 years ago which uses (+)-α-pinene as chiral starting material[5]. Commercial (+)-α-pinene is generally only 95–98% enantiomerically pure and would be expected to lead to products with a corresponding level of enantiomeric purity. Hence any psychotropic activity in the (3S,4S) series may have been due to the presence of variable amounts of the respective (3R,4R) enantiomer.

Materials and methods. The syntheses of compounds (**II**) and (**III**) will be reported separately. The starting material for (**II**) is enantiomerically pure 3-oxo-myrtenyl pivalate (**V**), m.p.



Structures of the natural tetrahydrocannabinols (delta-1-THC, **I**, and delta-6-THC, **IV**) and general indication of the synthetic route to the enantiomers of the 1,1-dimethylheptyl (DMH) homologs of 7-hydroxy-delta-6-tetrahydrocannabinol (compounds **II** and **III**).