crystalline bovine plasma albumin as standard. The specific activity of aconitase was expressed as units/mg of protein. The Student's t-test[9] was used to evaluate the significance of the differences between Li_2CO_3 treated and control mice.

Results and discussion. The mean intake of water or Li_2CO_3 solution during the period of the experiment was the same (5.9 ml/mouse/day) in both groups of mice. This volume is equivalent to 19.7 mg of Li_2CO_3/kg of body weight/day, which is in the range of the dosage used in manic-depressive syndrome[2].

The results are summarized in the Table. Administration of Li_2CO_3 does not influence the final body weights of the animals (t=1.098, $P<0.5$). On the other hand, an inhibition of the specific brain aconitase activity was observed in Li_2CO_3 treated mice. This inhibition was found statistically significant (t=2.611, $P<0.02$). Attempts to demonstrate an in vitro effect of Li^+ were negative. Addition of Li_2CO_3 up to 0.5 mg to the incubation system did not change brain aconitase activity.

It is interesting to emphasize that the Li^+ effect on mouse brain aconitase was observed only in vivo as well as the action of this ion on brain succinate dehydrogenase activity[6]. Certainly, further studies are necessary to define whether the effects of Li_2CO_3 on these enzymes have any connection with the clinical effects of the drug.

Résumé. Nous avons déterminé l'activité spécifique de l'aconitase cérébrale des souris après une période de 108 jours de traitement avec du lithium (Li_2CO_3). Une inhibition significative de l'enzyme à été observée. D'autre part, nous n'avons trouvé in vitro aucun effet du Li_2CO_3 sur l'aconitase cérébrale.

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Compound 48/80 Decreases Adenosine 3′5′-Monophosphate Formation in Rat Peritoneal Mast Cells[1]

Adenosine 3′5′-monophosphate (cAMP) has been shown to influence the release of many compounds from a variety of cell types. In the majority of cases, e.g. thyroid hormone[2], amylase in the parotid gland[3], ACTH[4], growth hormone[5] and calcitonin[6], the release process appears to be mediated by an increase in cAMP levels. In contrast, agents which raise cAMP levels inhibit the antigen-induced release of histamine from the leukocytes of allergic individuals[7,8] and also the release of histamine from rat peritoneal mast cells by compound 48/80[9,10]. These latter observations suggested that the releasing agents in question might lower cAMP levels in these cell types. This possibility was explored by examining the effect of compound 48/80 on cAMP formation in purified rat peritoneal mast cells. Compound 48/80 at a concentration sufficient to release 50 to 60% of the total histamine present in the cells decreased the formation of cAMP by approximately 50%.

Materials and methods. Peritoneal cells were removed from female Sprague-Dawley rats and mast cells purified by repeated centrifugation of the cells for short time intervals as previously described[11]. The final cell preparation consisted of 65–90% mast cells. The formation of cAMP from (^{3}H)adenine[12,13] was measured by the method of Krishna et al.[14]. Cells were preincubated with (^{3}H)adenine (30 μc/ml) for 1 h, washed twice, resuspended with and without compound 48/80 and incubated for 15 min.

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Effects of compound 48/80 on histamine release and cAMP formation in rat peritoneal cells

Expt	48/80 (γ/ml)	Time (min)	Histamine Release (%)	Inhibition of cAMP[a] formation (%)
1–6	1.0	15	55.0 ± 9.5[a] (n = 8)	56.4 ± 14.3[a,b] (n = 12)
5,6	0.2	15	16.0 ± 4.4 (n = 4)	30.7 ± 11.5 (n = 4)
	1.0	15	52.3 ± 12.8 (n = 4)	53.3 ± 12.3 (n = 4)
7	1.0	1/12	69	58
	1.0	1/2	64	52
	1.0	2	72	60

[a] Average ± S.D. [b] Experiments were done in duplicate. Inhibition was calculated against the average of 2 controls for each experiment. The average difference between control values in 6 separate experiments was 12%.

The incubation was terminated by adding 2 ml ice-cold medium and centrifuging the cells at 1600 $\times g$ for 1 min. Cells and supernatants were separated and the histamine in the supernatant fraction determined by the fluorometric method of SHORE[15] as modified by KREMZLER and WILSON[16] (15). Unlabeled cAMP (3 mg) and a tracer amount of (^{14}C) cAMP in 3 ml water were added to the cell pellets. The tubes were then heated in a boiling water bath for 3 min and a portion of the solution eluted from Dowex 50 columns (0.6 $\times$ 3.0 cm) with water. The cAMP containing fraction (6th and 7th ml) was treated with a mixture of barium hydroxide and zinc sulfate 3 times[14]. Portions of the final supernatants were counted in a Packard Instrument Company liquid scintillation counter using standard double-label counting techniques. The recovery of (^{3}H)cAMP was determined from the known (^{14}C)cAMP recovery. The conversion of incorporated radioactivity to cAMP in control flasks was approximately 0.5%. Total radioactivity retained by the cells was unaffected by compound 48/80.

The medium used throughout was composed of (m*M*): NaCl — 139; KCl — 6; $MgSO_4$ — 1.2; $CaCl_2$ — 2; dextrose — 10; N-*tris* (hydroxymethyl) methyl — 2-aminoethane sulfonic acid (TES) — 10, pH 7.4 and human serum albumin — 0.1%. All glassware that came into contact with the cells was siliconized (^{3}H) adenine, (62.1 mC/mg) and (^{14}C) cAMP, (0.11 mC/ml) were obtained from New England Nuclear Corp., Boston, Mass. and compound 48/80 was the gift of Burroughs-Wellcome Company.

Results and discussion. Compound 48/80 at a level of 1.0 γ/ml inhibited the formation of radioactive cAMP from precursor adenine by an average of 56% in 7 experiments (15 observation) (Table). The degree of inhibition correlated with both the concentration of 48/80 and the histamine released (exp. 5 and 6, Table). Attempts to demonstrate that the decrease in cAMP formation preceded the release of histamine were unsuccessful. At the earliest time interval that could be conveniently studied (5 sec) histamine release and the change in cAMP levels had both occurred. (Table, exp. 7).

The action of compound 48/80 on rat peritoneal mast cells has been studied extensively. It is known to release the histamine containing granules within seconds by a non-cytotoxic mechanism. The release process can be inhibited by metabolic inhibitors[17] but does not alter ATP levels within the cells[18,19]. The molecular mechanism of the action of compound 48/80 is nuclear. In the present study compound 48/80 was found to decrease the formation of cAMP from adenine in purified rat peritoneal mast cells. The decrease paralleled the 48/80 concentration and occurred at least as rapidly as did histamine release. Since increased cAMP levels inhibit histamine release from mast cells it is reasonable to suppose that decreased levels initiate the release process. Numerous releasing agents raise cAMP levels (see above). The present findings appear to represent the first case in which a releasing agent lowers cAMP formation in its target cells.

Zusammenfassung. Präparat 48/80 unterdrückt die Bildung von 3′, 5′-AMP aus Adenin in isolierten peritonealen Mastzellen. Es konnte keine zeitliche Verschiebung zwischen dieser Unterdrückung und der Histamin freisetzenden Wirkung von Präparat 48/80 festgestellt werden.

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Renal Tubular Reabsorption of Acetaminophen after Vasopressin Administration in Man

In the dog vasopressin has been shown to increase the renal tubular reabsorption of acetaminophen (paracetamol)[1], the major metabolite of phenacetin. Since the latter has for long been incriminated in the genesis of analgesic nephropathy[2], we have examined the effect of vasopressin on the reabsorption of acetaminophen from the tubular fluid in man.

Experiments were performed in 4 normal subjects, who were given a water load of 20 ml/kg at 09.00 h on the day of the experiment together with acetaminophen 30 mg/kg. Thereafter the state of overhydration was maintained constant by giving water equal in volume to urine passed. About 1$^1/_2$ h after the water load, urine collections were made at 5–10 min intervals and when urine flow was constant vasopressin 0.7 m units/kg was given i.v. Because of marked changes in urine flow, it is impossible during the resulting antidiuresis to measure standard renal clearances due to errors of dead space and flow. These errors, however, are identical for all urinary solutes and changes in the composition of the urine due solely to the removal of water should affect all solute concentrations to the same extent. Thus if, as urine flow falls the concentration of a urinary solute rises less than that of a glomerular substance; in this case creatinine, then the tubular reabsorption of that solute must have increased during the period of vasopressin activity.

The results are shown in the Table. Changes in the U/P concentration of a urinary solute during the antidiuretic period when urinary creatinine concentration was maximal are related to the mean of the U/P concentrations of that solute in the period immediately before giving vasopressin and in the period 80–120 min afterwards, when urine flow had returned to within 70% of control. This method of expression minimizes spontaneous changes in solute concentration unrelated to activity of the hormone. In all instances after giving vasopressin the U/P concentration of acetaminophen rose less than that of creatinine, with a mean increase of 44% (99% confidence limits 22% to 83%) that of creatinine. This increase was closely similar to that of urea (mean 39%) whose reabsorption is well known to increase during vasopressin activity[3].

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