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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U/P concentrations and concentration ratios following vasopressin administration

Subject	Experiment	Urine Flow		Creatinine		U/P concentrations				U/P concentration ratios	
		c[a]	v[c]	c	v	Urea c	Urea v	Acetaminophen c	Acetaminophen v	v/c urea / v/c creatinine	v/c acetaminophen / v/c creatinine
M.B.	1	8.0	0.39	16.8	409.1	8.1	46.2	2.10	12.10	0.23	0.23
M.B.	2	10.2	0.70	13.0	212.5	7.7	62.5	0.58	6.85	0.45	0.72
J.F.	1	8.6	0.43	13.7	203.2	6.7	24.9	1.82	8.73	0.25	0.32
J.F.	2	9.7	0.61	12.1	143.2	7.0	18.8	0.82	4.12	0.25	0.42
P	1	9.0	1.25	13.0	104.2	9.0	46.3	1.45	8.0	0.65	0.69
P	2	11.9	2.56	12.6	83.7	7.6	38.2	1.23	5.20	0.75	0.64
F.N.	1	8.7	0.66	14.2	315.8	7.2	69.0	3.02	17.60	0.43	0.26
mean[b]										0.39	0.44
99% confidence limits										0.20–0.78	0.22–0.83

Abbreviations: [a] C, control period data derived from the mean of the period immediately before giving vasopressin and in the period 80–120 min afterwards when urine flow had returned to within 70% of control. [b] V, vasopressin period when urinary creatinine concentration was maximal. [c] Mean and confidence limits derived from the $\log_{10}$ distribution.

The results indicate that vasopressin increases the tubular reabsorption of acetaminophen in man and would lead to its sequestration at high concentration in the renal papilla[4]. It is possible that acetaminophen at a sufficiently high concentration may itself have a direct toxic effect on the papilla. Alternatively it may play a part in the nephrotoxicity of phenacetin by its ability to potentiate the action of vasopressin[5] and thereby the toxicity of other metabolites of this drug, such as *p*-phenitidine.

Résumé. Nous avons comparé les concentrations U/P d'acétaminophène et de créatinine avant et après l'administration de vasopressine à l'homme. Nous avons constaté que l'hormone augmente la réabsorption tubulaire d'acétaminophène, ce qui peut être important dans la production de la néphropathie du type analgésique.

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Structure-Activity Relationship of the Cardenolide, with Special Reference to the Substituents and Configurations at C-14 and C-15

In previous communications, we reported: 1. 14-Deoxy-14βH-uzarigenin was about one-third as active as uzarigenin, indicating that the presence of 14β-hydroxyl group is not indispensable for the positive inotropic activity of cardenolides, 2. the introduction of an oxygen function to C-15 of digitoxigenin profoundly affected the inotropic activity in the following order: digitoxigenin (I) > 15β-hydroxydigitoxigenin (II) > 15-oxodigitoxigenin (III) > 15α-hydroxydigitoxigenin (IV) the last being practically inactive[1]. Extending these observations, six derivatives of 14-deoxy-14βH-digitoxigenin (V) and 14-deoxy-14β-chloro-digitoxigenin with oxygen functions at C-15 were examined and compared.

The compounds used in this experiment were digitoxigenin (I), 3β, 15β-dihydroxy-5β, 14β-card-20 (22)-enolide (VI)[2], 3β-hydroxy-15-oxo-5β, 14β-card-20 (22)-enolide (VII)[3], 3β, 15α-dihydroxy-5β, 14β-card-20 (22)-enolide (VIII)[2], 3β, 15β-dihydroxy-14-chloro-5β, 14β-card-20 (22)-enolide (IX)[2], 3β-hydroxy-15-oxo-14-chloro-5β, 14β-card-20 (22)-enolide (X)[2] and 3β, 15α-dihydroxy-14-chloro-5β, 14β-card-20 (22)-enolide (XI)[2]. Stock solutions were prepared with each compound dissolved in 70% ethanol in concentration of 1 mg/ml.

The inotropic activities of these compounds were tested with the isolated frog's heart (Straub's preparation), by the same method used in the previous papers[1,4]. The Straub's cannula contained 2 ml of Ringer's solution, the composition of which was: NaCl 111 m*M*, KCl 2.7 m*M*, $CaCl_2$ 1.8 m*M*, $NaHCO_3$ 1.2 or 18 m*M* and glucose 2.7 m*M*. When the solution contained 1.2 m*M* $NaHCO_3$, it was aerated with air. When it contained 18 m*M* $NaHCO_3$, aeration was made with 95% O_2 + 5% CO_2. The contraction of the heart was recorded with an isotonic lever. The heart was first made hypodynamic by reducing the concentration of calcium to 0.6 m*M*, $^1/_3$ of the normal, and then the effect of one of the compounds was tested in the following way.

The stock solutions were diluted with either distilled water or with the low Ca Ringer to desired concentrations before experiment. Starting from a subthreshold dose, a small amount (0.02–0.14 ml) of a diluted solution was added to the cannula every fifteen minutes, so that a stepwise increase in the cumulative concentration of the test compound was achieved, until the heart went into systolic contracture. The way of increase in the cumulative

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