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Quercetin enhances water transport in toad bladder

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Summary. A highly significant enhancement of the hydrosmotic actions both of vasopressin and of exogenous cAMP was seen in the presence of quercetin. The hypothesis is advanced that quercetin affects the intracellular coupling between Ca^{++} and cAMP.

Work from this laboratory has shown that some inhibitors of (Na+K)ATPase, e.g. harmaline and vanadate, affect water transport across amphibian epithelia[2-4]. This property, which is not shared by the classical Na pump inhibitor, ouabain, can be used to analyze fundamental aspects of epithelial transport, such as the coupling of Na and water flows, and the mechanism of the modulation of cell permeability by hormonal signals[2-4]. Along this line, we investigated the effects of the flavonoid quercetin, another inhibitor of (Na+K)ATPase[5]. We report here that quercetin quickly and reproducibly enhanced the transepithelial water flow (J_w), induced by vasopressin or by exogenous cAMP, in toad bladder.

Materials and methods. Tropical toads *(Bufo marinus)* were obtained from Charles P. Chase Co., Miami, Florida, USA, and kept in a terrarium with free access to water. The urinary bladders were exposed to a standard osmotic gradient of approximately 200 mosm/kg H_2O. The composition of the Ringer solution was (in mM): NaCl, 112; $MgSO_4$, 1; KH_2PO_4, 1.2; $KHCO_3$, 2; $CaCl_2$, 1; osmolality, 220 mosm/kg H_2O; pH was adjusted to 7.8. The serosal side of the bladder was bathed by full-strength Ringer and the mucosal side by the same solution diluted 10-fold. Transepithelial water fluxes were continuously monitored with automatic, volumetric techniques previously described[2,6,7]. The drugs used were: vasopressin (Pitressin, Parke-Davis), cyclic AMP (Sigma) and quercetin (Sigma). The latter was dissolved in dimethylsulfoxide (DMSO), so that the concentration of DMSO was 0.05% (v/v) for 10^{-4} M quercetin in the Ringer solution. Where applicable, results were expressed as mean ± SEM and the p-values obtained by means of the Student's t-test for paired data.

Results. Quercetin can induce by itself a hydrosmotic effect across toad bladder. Although this action was not seen in every experiment, the increase in J_w was quite conspicuous and sustained in some bladders, as shown in figure 1. In a series of 12 consecutive experiments J_w went from 0.02 ± 0.01 to 0.30 ± 0.12 $\mu l \cdot min^{-1} \cdot cm^{-2}$ ($p < 0.05$), on addition of 0.1 mM quercetin to the serosal medium. Regardless of whether the drug stimulated J_w or not, the subsequent addition of vasopressin revealed that the hormonal effect was not only present (fig. 1) but even appeared to be enhanced by quercetin. To test this point, a series of paired studies was carried out in quarter-bladders of the same animal. Quercetin did potentiate the action of vasopressin (fig. 2) as can be easily seen by inspection of the peak values of J_w and of the cumulative J_w-values shown at the right hand side of the same figure.

As a step towards localizing the site of action of quercetin, we studied next the interaction between the flavonoid and exogenous cAMP. The results are summarized on figure 3 and clearly show that quercetin also enhanced the hydrosmotic action of cAMP. Finally, we looked at the action of quercetin in bladders in which J_w had been previously stimulated by vasopressin or cAMP. The drug produced a further increase in J_w, the onset of which was very rapid (within 1 min, see left part of figure 3, lower recording). Comparison of the cumulative J_w-values during 30-min periods before and after the addition of quercetin gave the following results: a) 35.8 and 72.0 $\mu l \cdot cm^{-2}$, respectively, in bladders preexposed to supramaximal concentration of vasopressin (50 $mU \cdot ml^{-1}$, $N = 9$, $p < 0.01$); b) 15.7 and 52.2 $\mu l \cdot cm^{-2}$, respectively, in bladders pre-exposed to cAMP (5 mM, $N = 6$, $p < 0.01$).

Discussion. The hitherto unreported effects of quercetin on water transport appear to be unique. Most drugs known to interfere with vasopressin-induced water flow in toad blad-

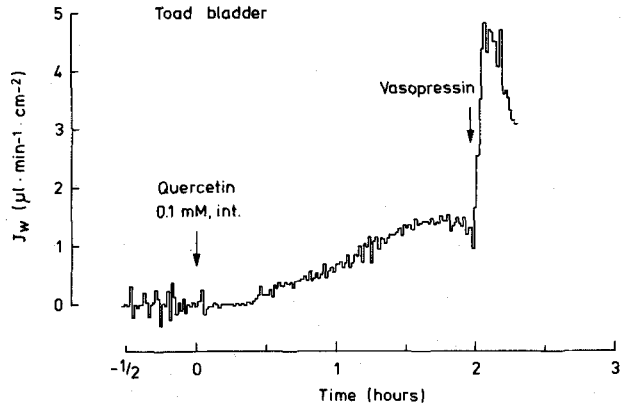

Figure 1. Stimulation of transepithelial osmosis (J_w) by quercetin added to the serosal (int.) medium bathing toad bladder. The presence of quercetin did not prevent a further increase in J_w by vasopressin (50 $mU \cdot ml^{-1}$).

der (e.g., lithium, vanadate, colchicine, cytochalasin B) have an inhibitory action[4,8-10]. It has been reported[11,12] that high concentrations of hydrazine (10–20 mM) potentiate the hydrosmotic action of vasopressin; however, hydrazine, unlike quercetin, does not enhance the response to cAMP.

It is of interest to discuss the effects of quercetin on Jw with reference to current concepts of the stimulus-effect coupling of vasopressin. The latter starts with the occupancy of specific receptors, the activation of adenylyl cyclase and the generation of cAMP. The final biological effect, i.e., the increase in water permeability, appears to result from a restructuring of the apical membrane, with formation of intramembrane-particle aggregates functioning as osmotic shunts across the lipid bilayer. The appearance of such aggregates, containing specific water channels, seems to depend not only on cAMP, but also on changes in intracellular Ca^{++} and in the microtubular-microfilament system[13-16]. The hydrosmotic effect of quercetin, whenever present (figs 1–3), very probably results from an elevation of intracellular cAMP due to a change in the activity of adenylyl cyclase and/or phosphodiesterase. An elevation of cAMP has been documented in Ehrlich ascites tumor cells exposed to quercetin[17]. Moreover, a similar explanation was advanced to account for the hydrosmotic effects, in toad skin, of psychotropic drugs such as harmaline, amitriptyline and chlorpromazine, all of which are also inhibitors of the Na pump[2,3,18]. It has been postulated that some kind of interaction between (Na+K)ATPase and adenylyl cyclase might lead to a rise in intracellular cAMP when the Na pump is inhibited[3,17,19], but direct evidence to support this attractive hypothesis is still lacking. On the other hand, a theophylline-like inhibition of phosphodiesterase by quercetin has been reported[20].

The quercetin-induced enhancement of the hydrosmotic actions both of exogenous cAMP and of supramaximal concentrations of vasopressin, strongly suggests that this flavonoid has at least one site of action beyond the generation of cAMP. 2 lines of evidence indicate that such a site is Ca^{++}-dependent, either directly or via calmodulin: a) recent reports showed that quercetin has a major effect on the transport of Ca^{++} across different cell membranes[21,22]; b) several late steps in the stimulus-effect coupling of

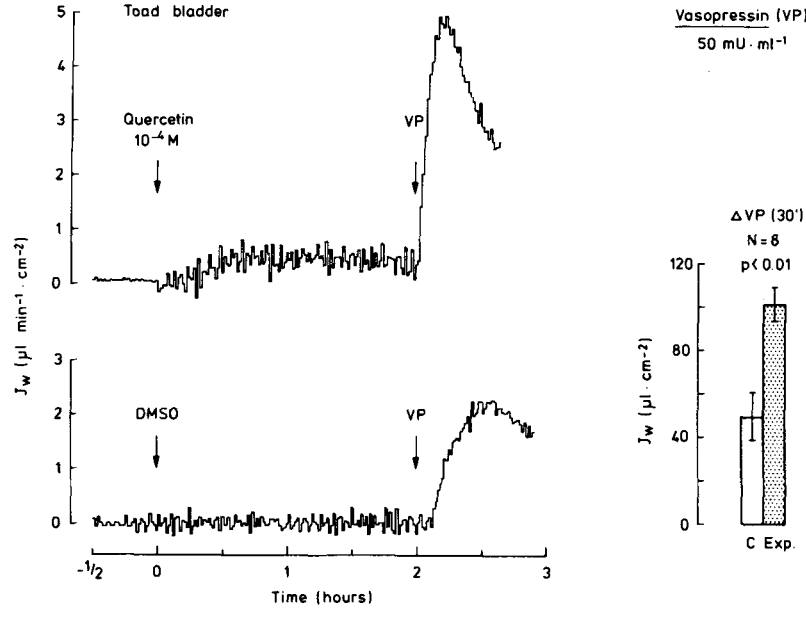

Figure 2. Comparison of vasopressin-induced water flows in paired quarter-bladders. Experimental tissues (Exp.) were pre-exposed to quercetin for 2 h. Control (C) and experimental tissues received the same amount of DMSO. On the right, the statistical analysis of cumulative (30-min) J_w-values (Δ VP), shows the significant enhancement of the hydrosmotic response to vasopressin induced by the flavonoid. On the left, the recording of a typical experiment is shown; note the conspicuous increase in peak J_w in the quarter-bladder exposed to quercetin.

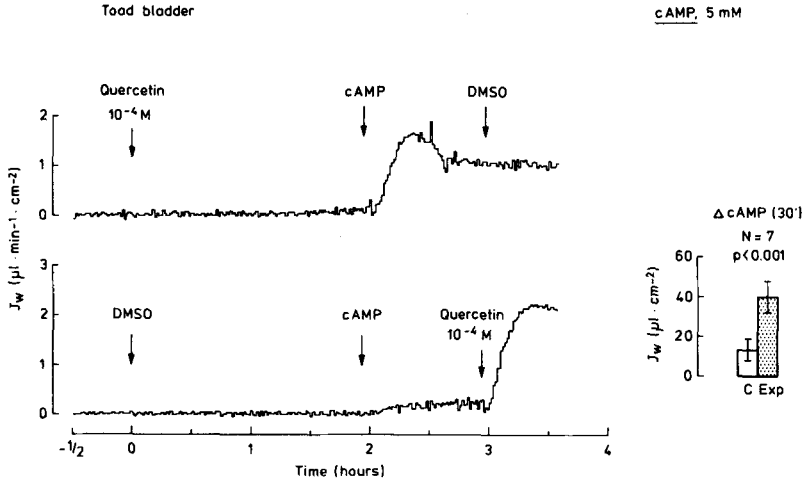

Figure 3. Effect of quercetin on cAMP-induced water flow. The design of the experiments was identical to that used for the vasopressin studies (see legend of fig. 2). The highly significant enhancement of the cAMP action (Δ cAMP, columns at the right side of the fig.) is also particularly evident in the example shown on the left. Note in this experiment the absence of hydrosmotic effect of quercetin, per se, and the very rapid increase in J_w when the flavonoid was added after exposure of the bladder to cAMP.

vasopressin are Ca^{++}-sensitive[23,24]. Thus, taking into account the mode of action of vasopressin, quercetin could modify either the number and/or the functional state of the osmotic shunts (intramembrane-particle aggregates) of the apical membrane.

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Refeeding of mice after fasting stimulates cell renewal in the gall bladder epithelium[1]

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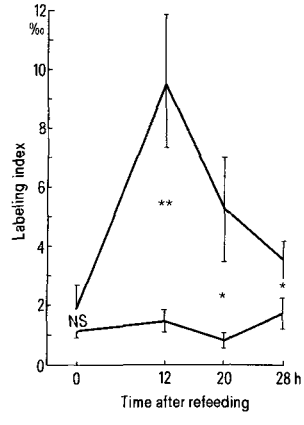
Summary. The effect of refeeding of mice after a fasting period on the uptake of 3H-thymidine and on mitotic activity in the gall bladder epithelium was studied by histoautoradiography. A significant increase in both DNA synthesis and mitotic activity was observed after 12 h of refeeding.

The gall bladder as well as the pancreas are well known target organs of cholecystokinin (CCK-PZ). Besides its effects on pancreatic secretion, on the muscular contraction of the gall bladder and on the secretion of glycoproteins by the gall bladder epithelium[3], this hormone exerts a significant growth promoting effect on both organs in the adult animal. Cholecystokinin and also caerulein, a synthetic decapeptide analogue, stimulate DNA synthesis[4,5] and increase total DNA content in the pancreas[6]. Recently, we have observed a potent stimulation of the DNA synthesis activity in the gall bladder epithelium of mice after acute administration of caerulein[7]. The physiological significance of this trophic effect remains unknown, however.

Feeding is one of the physiological stimuli for the release of gastrointestinal hormones, namely of cholecystokin. As far as we know, the influence of a meal on pancreatic or gall bladder growth has never been investigated. In the present study, the effect of feeding on cell proliferation in the murine gall bladder epithelium was studied by autoradiography.

Material and methods. 80 C57 black mice weighing 21-23 g were randomly subdivided into 2 equal groups. The animals in the 1st group were allowed free access to food after a 48-h period of fasting. The animals in the 2nd (control) group were allowed free access to food throughout the experiment without any preliminary fasting period. Ten animals from each group were killed simultaneously by neck dislocation at 0 h, 12 h, 20 h and 28 h, after refeeding the mice of the fasting group. The total food intake by each group was measured during these periods. The hours of killing were 12.00 h (0-h interval), 24.00 h (12-h interval), 08.00 h (20-h interval), and 16.00 h (28-h interval). All the animals were given an i.p. injection of 1 µCi/g b.wt of tritiated thymidine (Radiochemical Center, Mol, Belgium, sp. act. 16 Ci/mM) 1 h before killing. Cholecystectomy was performed after ligation of the cystic duct with



Mean labeling indices (± SEM) in the gall bladder epithelium of mice at intervals after refeeding and in control mice killed at the same hour. Labeling index values were higher after refeeding the animals: * $p < 0.05$; ** $p < 0.01$.