The purpose of this study was to characterize the role of ions other than Ca\(^{2+}\) in hepatic responses to α\(_1\)-adrenergic stimulation. We report that the α\(_1\)-adrenoceptor activation of hepatic functions is accompanied by extracellular acidification and an increase in intracellular pH. These effects are dependent on extracellular Na\(^+\) concentration and are inhibited by the Na\(^+\)/H\(^+\) antiporter blocker 5-(N-ethyl-N-isopropyl) amiloride under conditions that preclude antagonistic effects on agonist binding. Thus, the activation of plasma membrane Na\(^+\)/H\(^+\) exchange is an essential feature of the hepatic α\(_1\)-adrenoceptor-coupled signaling pathway.

The following observations indicate that the sustained hepatic α\(_1\)-adrenergic actions rely on a functional coupling between the plasma membrane Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) exchangers, resulting in the stimulation of Ca\(^{2+}\) influx. 1) Inhibition of the Na\(^+\)/K\(^+\)-ATPase does not prevent the α\(_1\)-adrenergic effects. However, α\(_1\)-adrenoceptor stimulation fails to induce intracellular alkalization and to acidify the extracellular medium in the absence of extracellular Ca\(^{2+}\). 2) A non-receptor-induced increase in intracellular Na\(^+\) concentration, caused by the ionophore monensin, stimulates Ca\(^{2+}\) influx and increases vascular resistance. 3) Inhibition of Na\(^+\)/Ca\(^{2+}\) exchange prevents, in a concentration-dependent manner, most of the α\(_1\)-agonist-induced responses. 4) The actions of Ca\(^{2+}\)-mobilizing vasoactive peptide receptors or α\(_2\)-adrenoceptors, which produce neither sustained extracellular acidification nor release of Ca\(^{2+}\), are insensitive to Na\(^+\)/H\(^+\) exchange blockers.

Functional Coupling of Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) Exchangers in the α\(_1\)-Adrenoreceptor-mediated Activation of Hepatic Metabolism*

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The activation of hepatic α\(_1\)-adrenoceptors produces biphasic changes in ion fluxes across the plasma membrane, the significance of which has not yet been ascertained (1–9). The role of Ca\(^{2+}\) in the receptor-mediated activation of hepatic metabolism has been thoroughly studied (4, 5). The acute rise in the free cytosolic Ca\(^{2+}\) concentration that follows the receptor-ligand interaction may be responsible for the activation of certain enzymes and for the acute and transient release of Ca\(^{2+}\) to the extracellular medium. However, no definite explanation has yet been offered about the significance of the α\(_1\)-adrenoceptor-induced sustained release of Ca\(^{2+}\) (2, 3). The Ca\(^{2+}\)-mobilizing agents phenylephrine and vasopressin produce similar hepatic metabolic responses, even though their effects are accompanied by net sustained release and uptake of Ca\(^{2+}\), respectively. Recent work indicates that receptor-induced changes in net Ca\(^{2+}\) movements across the hepatic plasma membrane can be dissociated from their metabolic actions (6). The latter, taken together with previous reports that indicate that α\(_1\)-adrenoceptor activation can elicit characteristic responses in Ca\(^{2+}\)-loaded cells (7) or in the presence of the Ca\(^{2+}\)-ionophore A23187 (8, 9), suggests that elucidation of the hepatic α\(_1\)-adrenoceptor signaling pathway requires explanations other than those based merely on perturbations of Ca\(^{2+}\) homeostasis.

α\(_1\)-Adrenoreceptor activation produces intracellular alkalization in several types of cells (10–13), indicating that the electroneutral Na\(^+\)/H\(^+\) antiporter is activated. In isolated liver cells, data suggesting the agonist-induced activation of Na\(^+\)/K\(^+\) and Na\(^+\)/H\(^+\) exchange have been reported (14). However, the physiological significance of those observations has never been ascertained, presumably because previous reports had shown that exchange of Na\(^+\) between tissue and medium was not involved in the Ca\(^{2+}\)-extruding mechanisms of liver cells (15).

Unlike other Ca\(^{2+}\)-mobilizing receptors, a characteristic feature of the hepatic α\(_1\)-adrenoceptors is their ability to elicit sustained release of Ca\(^{2+}\) and sustained metabolic stimulation (6). In this study, we report that the α\(_1\)-adrenoceptor-mediated sustained activation of hepatic functions is dependent on extracellular Na\(^+\) and Ca\(^{2+}\) and is distinctly inhibited by Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) exchange blockers. We propose that during hepatic α\(_1\)-adrenoreceptor activation, a functional coupling between the plasma membrane Na\(^+\)/H\(^+\) and Na\(^+\)/Ca\(^{2+}\) exchangers leads to enhanced Ca\(^{2+}\) influx and stimulation of Ca\(^{2+}\)-dependent processes.

EXPERIMENTAL PROCEDURES

Animals—Male Wistar rats (180–220 g in body weight) were used in all experiments. The animals were maintained under controlled conditions of light and temperature and free access to food and water. When indicated, the animals were starved for 48 h before their experimental use.

Liver Perfusion and Isolation of Liver Cells—Livers were perfused in a nonrecirculating system with Krebs-Ringer bicarbonate buffer according to previously described procedures (16, 17). Substrates were administered diluted in the perfusion medium, and agonists were infused into the portal vein.

Portal pressure, p\(_{OP}\), p\(_{K}\), p\(_{Ca}\), and pH of the effluent perfusate were continuously monitored in all experiments. The amplification of signals from each sensor as well as their digital conversion and computer analysis were carried out as previously described (6). Liver cells
Fig. 1. Role of extracellular Na⁺ or Ca²⁺ on α₁-adrenoceptor-induced hepatic responses. Livers from 48-h starved rats were perfused in a nonrecirculating system with Krebs-Ringer bicarbonate buffer at 36.5 °C and a flow rate of 30 ml/min. After an equilibration time of 30 min to attain steady rates of O₂ consumption, in the presence of the indicated concentrations of extracellular Na⁺, phenylephrine (PE) was administered diluted in the medium. The osmolarity of the medium was maintained by substitution of choline with sodium. Details on data collection and processing are described under "Experimental Procedures." Each experiment was repeated four to six times, obtaining reproducible results, and data from representative experiments are shown. The average absolute values ± S.E. obtained in control livers and livers perfused with 70 mm Na⁺, 35 mm Na⁺, or 50 μm Ca²⁺ were, respectively, as follows: oxygen uptake, 869 ± 31, 800 ± 50, 737 ± 56, and 826 ± 27 microatoms (μat)/100 g of body weight (B.Wt./h); pH, 7.326 ± 0.024, 7.38 ± 0.03, 7.365 ± 0.015, and 7.236 ± 0.031 units; [Ca²⁺], 1.097 ± 0.027, 1.000 ± 0.020, 1.298 ± 0.015, and 0.051 ± 0.001 μm; and glucose production, 15 ± 1, 20 ± 2, 15 ± 3, and 10 ± 1.3 μm/100 g of body weight/h.

RESULTS AND DISCUSSION

Influence of Extracellular Na⁺ or Ca²⁺ on α₁-Adrenergic Receptor-induced Activation of Hepatic Metabolism: Role of Na⁺/H⁺ Exchanger—Fig. 1 (left panels) shows that, in agreement with previous reports (2, 3, 6, 9, 22), the α₁-agonist phenylephrine produced biphasic stimulation of respiration, portal pressure, glucose mobilization, and H⁺ and Ca²⁺ efflux. The degree of extracellular acidification varies according to the nutritional status and the gluconeogenic substrates used (23). The cotransport of H⁺ with lactate (24) could be responsible for the α₁-agonist-induced extracellular acidification. However, in livers from fed animals, phenylephrine produced a decrease in the extracellular pH of 0.065 ± 0.007 unit (n = 10) and a mean lactate production of 74 ± 17 μmol/100 g of body weight/h (n = 10), whereas in starved livers, the change in pH was −0.025 ± 0.005 (n = 10), although the lactate output was <5 μmol/100 g of body weight/h. Thus, the α₁-adrenergic receptor-induced extracellular acidification cannot be accounted for by the cotransport of H⁺ with lactate.

The α₁-adrenergoreceptor-induced activation of hepatic functions depends on extracellular Na⁺ and Ca²⁺ (Fig. 1, middle and right panels). The extracellular Na⁺ dependence of the α₁-adrenergic receptor-induced extracellular acidification suggested that the Na⁺/H⁺ exchanger was activated. We tested this possibility by studying the effect of EIPA, a specific inhibitor of Na⁺/H⁺ exchange (25), on the α₁-adrenoceptor-mediated responses. EIPA prevented all the α₁-agonist-induced effects in a concentration-dependent manner (Fig. 2).

α₁-Adrenergic stimulation failed to produce a sustained release of Ca²⁺ at low extracellular Ca²⁺ concentration (Fig. 1, right panels), as has been previously observed (1, 2). This phenomenon has been assumed to reflect the lack of adequate Ca²⁺ influx for refilling the intracellular Ca²⁺ stores. Without physiological concentrations of extracellular Ca²⁺, α₁-adrenergic...
stimulation also failed to acidify the extracellular medium and to increase vascular resistance (Fig. 1). Thus, the $\alpha_1$-adrenoceptor-induced stimulation of Na$^+/H^+$ exchange seems to require a concomitant influx of Ca$^{2+}$. The extracellular Na$^+$ and Ca$^{2+}$ dependence of the $\alpha_1$-adrenergic actions suggested a possible role of the Na$^+$/Ca$^{2+}$ exchanger in the adrenergic actions. Figs. 3 and 4 show that benzamil, an inhibitor of Na$^+$/Ca$^{2+}$ exchange (26), at a concentration of 1 $\mu$M, which precludes significant action on the Na$^+$/H$^+$ exchanger, inhibited all the $\alpha_1$-adrenergic receptor-induced hepatic responses. The effect was more pronounced over the sustained phase of $\alpha_1$-adrenergic stimulation. The sustained phase of Ca$^{2+}$ release was more sensitive to benzamil than to other parameters (Fig. 3). This observation agrees with data in Fig. 1 showing that $\alpha_1$-adrenoceptor-induced sustained release of Ca$^{2+}$ demands a normal rate of influx of this ion. The decreased effectiveness of $\alpha_1$-adrenoceptor activation in stimulating glucose mobilization, respiration, and vascular resistance most probably reflects the decreased rate of Ca$^{2+}$ influx (Fig. 3). The smaller extracellular acidification (Fig. 3) may be caused, at least in part, by the diminished rate of glycogenolysis and, consequently, the smaller rate of lactate (and H$^+$) output. In livers from starved rats, the carbohydrate stores are scarce, and the rate of lactate output is almost negligible. In this case, inhibition of Na$^+$/Ca$^{2+}$ exchange prevented the $\alpha_1$-adrenergic receptor-induced release of Ca$^{2+}$, but significant extracellular acidification was still detected (Fig. 4). This observation suggests that the inhibition of extracellular acidification in fed livers (Fig. 3) may be related to the decreased rate of carbohydrate mobilization (lactate and H$^+$ output).

EIPA, Benzamil, and Na$^+$-induced Changes in $\alpha_1$-Adrenoceptor Ligand Affinity—The significance of the EIPA or benzamil actions could be questionable in view of previous reports indicating that in isolated plasma membranes, amiloride or its 5-amino substituted derivatives inhibit competitively the binding of agonists to $\alpha_1$-adrenergic receptors (27). On the other hand, monovalent cations regulate allosterically the binding of ligands to G protein-coupled receptors (28, 29). An aspartate residue conserved among G protein receptors seems to be responsible for the allosteric regulation by Na$^+$(30). Data in Fig. 5 show that a 10-fold increase in the $\alpha_1$-agonist concentration failed to reverse the inhibitory actions of extracellular Na$^+$ removal, EIPA, or benzamil on the $\alpha_1$-adrenergic receptor-mediated responses. Thus, the effects of extracellular [Na$^+$] and Na$^+$/H$^+$ or Na$^+$/Ca$^{2+}$ exchange blockers on the $\alpha_1$-adrenoceptor-mediated responses were not only the result of ligand affinity changes. The idea that Na$^+$/H$^+$ exchange blockers might inhibit $\alpha_1$-adrenergic actions by mechanisms other than competing with $\alpha_1$-agonist binding is implicit in a previous report of Lesburg et al. (25) in that phenylephrine dose responses of vascular smooth muscle contraction were shifted downward and to the right by amiloride. Although that observation was interpreted as the result of noncompetitive antagonism of $\alpha_1$-adrenoceptor ligand binding (25), an alternative explanation might be that activation of Na$^+$/H$^+$ exchange is involved in $\alpha_1$-agonist-activated muscle contraction.

Portal pressure showed a significant recovery upon increases in agonist concentration in livers perfused with EIPA or low Na$^+$-containing medium (Fig. 5). This suggests that, unlike the other parameters, the $\alpha_1$-adrenoceptor stimulation of vascular smooth muscle contraction does not rely on the activity of the Na$^+$/H$^+$ exchanger.

Effect of Perturbing Na$^+$ Transmembrane Gradient on Hepatic $\alpha_1$-Adrenoceptor-mediated Responses—Inhibition of the Na$^+$/K$^+$ pump by ouabain (Fig. 6) did not alter the hepatic responses to phenylephrine, even though in parallel experiments carried out in liver cells, other parameters were found to be sensitive to lower concentrations of the inhibitor (data not shown). This observation suggests that the Na$^+$ taken up during $\alpha_1$-adrenergic receptor activation may exit the cell by means
The result of intracellular alkalinization or the enhanced Ca²⁺ cannot be ascertained whether the responses to monensin were decreasing [Ca²⁺] requirements (32). However, in other types of influx. Increased pH seems to enhance myofibrillar tension by cells (33-35), the artificial increasing of intracellular pH did not reproduce the agonist-induced effects.

Other than Na⁺/K⁺ exchange. The extracellular Ca²⁺ dependence (Fig. 1) and the inhibition by benzamil (Figs. 3 and 4) suggest that during α₁-adrenoceptor activation, intracellular Na⁺ may be exchanged for Ca²⁺.

The activation of Na⁺/H⁺ exchange (and therefore intracellular alkalization) seems to be a condition necessary for hepatic α₁-adrenergic stimulation (Fig. 2). Thus, one could ask whether non-receptor-induced intracellular alkalization is sufficient signal to activate liver functions. The cationophore monensin promotes Na⁺ cellular entry and H⁺ efflux (31). In isolated hepatocytes, monensin produced a significant elevation of intracellular pH (data not shown). Therefore, the application of monensin to perfused livers should mimic the α₁-adrenoceptor-mediated effects coupled to the activation of the Na⁺/H⁺ exchanger. Fig. 7 (left panels) shows that monensin produced a rapid fall in the extracellular pH that lasted as long as its application. This effect was paralleled by increased glucose mobilization and portal pressure. However, unlike phenylephrine, monensin produced a net influx of Ca²⁺. It also produced a sustained release of K⁺ of higher intensity than the α₁-receptors (33-35), the artificial increasing of intracellular pH did not reproduce the agonist-induced effects.

Fig. 7 (right panels) also shows that monensin did not prevent the α₁-adrenoceptor-induced extracellular acidification, glucose mobilization, release of Ca²⁺, and increase in vascular resistance. The only significant differences with the control without monensin were a pronounced influx of K⁺ and a smaller increase in portal pressure (Figs. 1 and 7, upper right panels). The additivity of monensin and α₁-adrenoceptor-mediated actions most probably reflects differences in their mechanism of action. The ability of α₁-adrenergic agonists to elicit hepatic responses in the presence of monensin seems to indicate that intracellular alkalosis is not a primary event in the signaling pathway. The additivity of monensin and phenylephrine actions, in spite of the remarkable stimulation of Ca²⁺ influx (Fig. 7), also adds further support to the operation of a Ca²⁺-independent α₁-adrenoceptor signaling pathway(s) (8, 9).

Specificity of α₁-Adrenergic Receptor-induced Stimulation of Na⁺/H⁺ Exchange—Fig. 8 shows that, unlike the α₁-adrenergic agonists, the stimulation of a vasoactive peptide receptor like vasopressin elicits increases in respiration and gluconeogenesis accompanied only by acute and transient release of H⁺ and Ca²⁺. A burst of K⁺ release was also observed starting immediately after the acute phase of H⁺ and Ca²⁺ release, which lasted for ~10 min. Inhibition of the Na⁺/H⁺ exchanger did not alter the ability of this Ca²⁺-mobilizing agonist to increase energy production and to stimulate gluconeogenesis. The only apparent effects of blocking Na⁺/H⁺ exchange were a smaller K⁺ release and a lack of sustained influx of Ca²⁺.

The α₂-adrenergic receptors account for ~20% of the total number of hepatic adrenoceptors, and their physiological role is still unclear (36). Unlike the α₁-adrenoceptors, the stimulation of α₂-receptors produces no detectable release of Ca²⁺.
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**Fig. 5.** Effect of increasing concentration of α₁-agonist on inhibition of α₁-adrenoceptor-induced responses produced by EIPA, benzamil, or low extracellular Na⁺. Livers from 48-h starved rats were allowed to equilibrate until steady rates of oxygen consumption were attained, and then phenylephrine (PE), EIPA, or benzamil was added sequentially for the periods of time and concentrations indicated by the boxes. The experiment was repeated five times, obtaining similar results, and a representative experiment is presented. The average absolute values ± S.E. obtained in control livers are described in the legend to Fig. 1. In the absence of extracellular Na⁺, they were as follows: oxygen uptake, 712 ± 37 microatoms (μat)/100 g of body weight (B. Wt.)/h; pH, 7.357 ± 0.062 units; [Ca²⁺], 1.295 ± 0.009 mM; and glucose production, 12 ± 7.5 μmol/100 g of body weight/h.

**Fig. 6.** Effect of ouabain inhibition of Na⁺/K⁺-ATPase on hepatic responses to α₁-adrenergic stimulation. Livers from 48-h starved rats were allowed to equilibrate until steady rates of oxygen consumption were attained, and then 1 mM pyruvate was added. Ouabain and phenylephrine (PE) were added sequentially for the periods of time and concentrations indicated by the boxes. The experiment was repeated five times, obtaining reproducible results, and a representative experiment is presented. The average absolute values ± S.E. obtained immediately before the addition of the agonist in the absence and presence of ouabain were, respectively, as follows: oxygen uptake, 1081 ± 42 and 1037 ± 24 microatoms (μat)/100 g of body weight (B. Wt.)/h; pH, 7.305 ± 0.025 and 7.410 ± 0.07 units; [Ca²⁺], 1.005 ± 0.001 and 1.006 ± 0.002 mM; and glucose production, 89 ± 7 and 80 ± 9 μmol/100 g of body weight/h.
and only mild changes in respiration and vascular resistance. The α₂-adrenergic agonist clonidine produced no detectable pH changes in the outflow perfusate (data not shown) in the presence of the α₁-antagonist prazosin at a concentration (10⁻¹⁰ M) that produces an almost complete inhibition of ligand binding to α₁-adrenergic receptors and phosphorylase activation. Therefore, the stimulation of the hepatic Na⁺/H⁺ antiporter appears to be distinctly associated with the α₁-type receptors.

α₁-Adrenergic Receptor-induced Changes in Intracellular pH—Further evidence in favor of the α₁-adrenergic activation of the hepatic Na⁺/H⁺ exchanger was obtained by measuring the agonist-induced intracellular pH changes. Fig. 9 shows that α₁-adrenergic stimulation of liver cells elicited intracellular alkalosis. In agreement with the observations made in the intact liver (Fig. 1), α₁-adrenergic receptor activation failed to increase intracellular pH in the absence of a physiological extracellular concentration of Na⁺. Moreover, 1 µM EIPA prevented the α₁-adrenergic receptor-induced alkalization of a 10-fold molar excess of α₁-agonist (Fig. 9). α₁-Adrenergic stimulation failed also to elicit intracellular alkalosis in the absence of extracellular Ca²⁺ (Fig. 9). This observation is consonant with data obtained in perfused liver (Figs. 1, 3, and 4) and provides further evidence that α₁-adrenergic receptor-coupled activation of Na⁺/H⁺ exchange requires normal rates of Ca²⁺ influx, or perhaps, a physiological concentration of extracellular Ca²⁺ may be required to be efficiently exchanged for intracellular Na⁺.

Significance of Na⁺/H⁺ Exchange Activation in α₁-Adrenergic Activation of Hepatic Metabolism: Functional Coupling of Na⁺/H⁺ and Na⁺/Ca²⁺ Exchangers—The α₁-adrenergic receptor-induced intracellular alkalization (Fig. 9), the extracellular Na⁺ concentration dependence of the pH changes (Figs. 1 and 9), and the inhibition of the hepatic responses by Na⁺/H⁺ exchange blockers (Fig. 2) indicate that activation of the Na⁺/H⁺ antiporter is a condition necessary for the hepatic α₁-adrenergic receptor to exert its actions and raise the question of its relationship to the metabolic response. The Na⁺/H⁺ antiporter appears to play important roles not only in maintaining intracellular pH and cell volume, but also in the action of mitogenic growth factors and in the cellular response to activating agents (33, 37, 38). In rat liver, this antiporter seems to be quiescent (Fig. 2), and α₁-adrenergic receptor activation stimulates its activity.

A diverse array of hepatic responses to α₁-adrenergic receptor activation, including energy production, metabolic activity, and ion fluxes, are inhibited by blocking Na⁺/H⁺ exchange. This suggests that activation of the antiporter is associated with an early common step in the signal transduction pathway. Phosphorylation of the transporter by either tyrosine kinases or protein kinase C has been associated with its activation (37). Most probably, the activation of the antiporter is associated with protein kinase C activity since non-receptor activation of hepatic protein kinase C produces extracellular acidification, and the α₁-adrenergic receptor-induced extracellular acidification is prevented by protein kinase C inhibitors (39).

Activation of the Na⁺/H⁺ exchanger does not seem to be sufficient signal to elicit a definite metabolic pattern of response.

Fig. 7. Effect of cationophore monensin on basal and α₁-adrenergic receptor activation of hepatic functions. Livers from rats fed ad libitum were allowed to equilibrate for =30 min until steady rates of oxygen consumption were attained, and then monensin was infused into the portal vein, for the periods of time indicated by the boxes to attain a concentration of 12.5 or 25 µM. Phenylephrine (PE) was administered diluted in the medium. The experiments were performed four to five times, obtaining reproducible results, and representative experiments are shown. The average absolute values ± S.E. obtained before monensin addition were as follows: oxygen uptake, 954 ± 40 microatoms (µat)/100 g of body weight (B.Wt.)/h; pH, 7.244 ± 0.027 units; [Ca²⁺], 1.000 ± 0.001 mM; and glucose production, 350 ± 29 µmol/100 g of body weight/h.
Na+/H+ exchange did not prevent the vasopressin-induced stimulation of gluconeogenesis (Fig. 8), although this hormone stimulates the Na+/H+ exchanger (42). In rat proximal nephrons, \(\alpha_1\)-as well as \(\alpha_2\)-adrenergic agonists increase the intracellular pH, but only \(\alpha_1\)-agonists are capable of stimulating gluconeogenesis and ammoniagenesis (43). In contrast, parathyroid hormone stimulates gluconeogenesis and ammoniagenesis in spite of its inhibitory action on Na+/H+ exchange (44). Therefore, although intracellular pH variations may cause rate changes in numerous enzymatic reactions, further work will be needed to elucidate the precise significance of these changes in the \(\alpha_1\)-adrenoceptor-mediated actions.

Stimulation of Na+/H+ exchange might also act independently of its effect on intracellular pH. The observation that blocking the Na+/Ca2+ exchanger perturbed the \(\alpha_1\)-adrenergic responses (Figs. 3 and 4) suggests that \(\alpha_1\)-receptor-mediated Ca2+ influx occurs through this exchanger. With few exceptions, the Na+/Ca2+ exchanger works in the Ca2+ extrusion mode (45); however, operation in the reverse mode has been observed by altering the Na+ gradient across the plasma membrane (46). It is worth noting that in excitable tissues, the \(\alpha_1\)-adrenergic receptor-induced intracellular alkalinization does not depend on extracellular Ca2+ (47, 48). Thus, the external Ca2+ dependence of the Na+/H+ exchanger seems to be a peculiarity of liver cells.

Exchange of internal Na+ for extracellular Ca2+ would explain the \(\alpha_1\)-adrenoceptor-induced intracellular alkalinization (49). It is worth noting that in excitable tissues, the \(\alpha_1\)-adrenergic receptor-induced intracellular alkalinization does not depend on extracellular Ca2+; however, the external Ca2+ dependence of the Na+/H+ exchanger seems to be a peculiarity of liver cells. Exchange of internal Na+ for extracellular Ca2+ would explain not only the extracellular Ca2+ dependence (Figs. 1, 3, 4, and 9), but also the lack of detectable effects of Na+/K+ pump inhibition on the \(\alpha_1\)-adrenoceptor-induced responses (Fig. 6).

In summary, the following observations support the proposal that the functional coupling of the Na+/H+ and Na+/Ca2+ exchangers is a distinct feature of sustained hepatic \(\alpha_1\)-adrenoceptor activation. 1) The sustained \(\alpha_1\)-adrenoceptor-mediated effects were dependent on extracellular Ca2+. 2) Non-receptor stimulation of Na+ influx by the catonophore monensin enhanced Ca2+ influx (Fig. 7). 3) The \(\alpha_1\)-adrenoceptor responses were sensitive to Na+/Ca2+ exchange blockers (Figs. 3 and 4). The \(\alpha_1\)-adrenoceptor-inhibited sustained effects on Ca2+ release and glycogenolysis, which are extracellular Ca2+-dependent processes, were also the most sensitive to Na+/Ca2+ exchange blockers. 4) Activation of \(\alpha_2\)-adrenoreceptors by clonidine produced neither significant extracellular acidification nor stimulation of Ca2+ release.

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