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Activation of Sodium Transport and Intracellular Sodium Lowering by the Neuroleptic Drug Chlorpromazine

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ABSTRACT. Chlorpromazine (CPZ), a commonly used antipsychotic drug, at high concentration was found to reduce significantly the sodium content of both rat (*Rattus norvegicus*) and toad (*Bufo marinus*) liver cells. This reduction in intracellular sodium was demonstrated using $^{22}Na^+$ flux and measurement of cell sodium content. The results suggest that the sodium-lowering effect of CPZ stemmed from a stimulation of sodium transport rather than from an inhibition of sodium influx (i.e., sodium channels), cell damage, or Na^+:Na^+ exchange. CPZ was found to interfere with the binding of ouabain to the sodium pump, although a simple reduction in sodium pump inhibition did not account for the sodium-lowering effect. CPZ was able to negate the effects of monensin, a sodium ionophore, suggesting a substantial capacity to activate sodium transport. The intracellular sodium-lowering action of CPZ through the activation of sodium transport represents a new property previously undescribed for this drug. BIOCHEM PHARMACOL **54**;2:275–281, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. liver cells; $^{22}Na^+$; sodium pump; ouabain; monensin; phenothiazines; *Rattus norvegicus*; *Bufo marinus*; sodium transport; active transport

CPZ† (i.e. Largactil®/Thorazine) is the best known and most commonly used compound of the phenothiazine group. A neuroleptic agent, CPZ is widely used as an antipsychotic. It is also used as a post-operative "sedative," in the alleviation of intractable hiccups and in the control of vomiting. The broad range and frequent use of this drug have led to many investigations into its cellular basis of action. Such studies have shown that CPZ has the ability to block dopamine (primarily D_2, directly linked to its antipsychotic effect), histamine, α-adreno, muscarinic, and 5-hydroxytryptamine receptors [1, 2]. It has also been reported to block sodium, calcium, and potassium channels [3–5] and inhibit an array of enzyme activities including both Na^+, K^+-ATPase [6] and Ca^{2+}-ATPase [7–10]. Accordingly, its multiplicity of cellular actions corresponds with a large number of adverse reactions, including hypotension [11], decreased thermal sensitivity [12], and considerable weight gain [13–15].

This present study was undertaken initially to see if the weight gain associated with the use of CPZ might be due to changes in the ionic permeability of cell membranes (i.e. plasma lemma). Since CPZ inhibits various ion channels [5, 16, 17], a decrease in ion permeability of the cell membrane could decrease the requirement for active transport and energy turnover associated with restabilizing ion gradients. Therefore, increases in weight gain may be due to reduced substrate turnover, from energy savings associated with reduced ionic permeabilities, and the consequent reduction in the cost of active transport [18]. However, subsequent to carrying out these experiments, the results suggested that, rather than inhibiting sodium movement, CPZ showed significant activation of sodium transport. This activation was a novel property previously undescribed for this or any other drug and became the primary area examined in this study.

To investigate the cellular action of CPZ on ionic permeability, short-term cultured liver cells were used from two disparate species to demonstrate common cellular actions. Liver cell monolayers were used because: (i) large numbers of cells can be isolated and prepared from a single animal, allowing for multiple drug treatments on the same cell population, and (ii) it is a familiar [18] and well characterized preparation, having been used for similar experiments, including a previous study on the effects of CPZ on ionic permeability and active transport [6].

MATERIALS AND METHODS

Chemicals

Collagenase (Type 1) was from the Worthington Biochemical Corp. (Freehold, NJ, U.S.A.). Dulbecco's modified Eagle's medium and HEPES (cell culture grade) were from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Other cell culture chemicals (serum, amino acids, and antibiotics) were purchased from Life Technologies (Melbourne, Australia). Lux Thermanox® coverslips were purchased from Trace Scientific (Sydney, Australia). Soluene 100® and

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† *Abbreviations:* CPZ, chlorpromazine.
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scintillation fluid were purchased from Canberra Packard (Zurich, Switzerland). Radioactive $^{22}NaCl$ (99.9% pure, specific activity 831 mCi/mg at 17.73 mCi/mL) was from DuPont (Boston, MA, U.S.A.). Ouabain, monensin, and CPZ were all purchased from Sigma. All other reagents used were of analytical grade.

Animal Species

Isolated liver cells were prepared from two species. The first species used was the laboratory rat (*Rattus norvegicus*, Sprague–Dawley strain, aged 6–14 weeks, body weight range 150–600 g). The second species used was the Cane toad (*Bufo marinus*, 70–150 g). Rats were obtained from the university rat colony and toads from a commercial supplier. Animals were maintained under a 12:12 hr light:dark photoperiod at 22° with free access to food and water. All animals appeared to be in good health at the time of the experiment.

Isolation and Short-Term Culture of Liver Cells

Methods relating to the isolation and short-term culture of liver cells were as previously described [18]. Briefly, animals were anaesthetized and heparinized (Nembutal®, 10–60 mg/kg, and 700 U of heparin for *R. norvegicus*, and tricaine methanesulfonate {MS222}, 0.5% at pH 7.4, and 500 U of heparin for *B. marinus*), and the hepatic portal vein was cannulated. Cannulated livers were cleared of blood, using a non-recirculating carbogenated (95% O_2:5% CO_2) wash solution (in mM: NaCl, 137; KCl, 5.4; $MgSO_4$, 0.8; Na_2HPO_4, 0.85; KH_2PO_4, 0.15; $NaHCO_3$, 25; glucose, 15; phenol red, 0.001%, pH 7.4) and then "digested" for 20 (rat) or 45 (toad) min via a recirculating enzyme solution (as for wash solution with 1 mM $CaCl_2$ and 0.05 to 0.1% collagenase enzyme). Perfusion rates varied from 13 mL/min for the toad to 30 mL/min for the rat. Perfusion rate and time were matched to produce similar total volumes of perfusate being circulated through the livers of both species. Digested livers were teased apart gently to release the isolated cells. Cell preparations were washed through 200 μm nylon gauze and centrifuged twice at 50 *g* for 5 min. Cell pellets were resuspended in Dulbecco's modified Eagle's medium (20% newborn calf serum, non-essential amino acids, and penicillin, streptomycin, and gentamycin at 50, 50, and 20 mg/L, respectively). Liver cells were plated onto collagen-coated (rat tail collagen prepared as previously described [19]) plastic Thermanox® coverslips (24 × 32 mm) and incubated overnight at 25° in a 95% O_2:5% CO_2 environment. Preparations routinely had viabilities of 85% or higher.

Measurement of Liver Cell Membrane Passive Permeability

The influx of $^{22}Na^+$ into liver cells was measured (as we have described previously [18]) by incubating the liver cell monolayers in a HEPES-buffered balanced electrolyte medium (in mM: NaCl, 150; $MgSO_4$, 0.8; $CaSO_4$, 1.2; K_2HPO_4, 0.8; KH_2PO_4, 0.14; HEPES, 10, pH 7.4, using KOH to raise the K^+ level to 5 mM) with $^{22}Na^+$ (at 0.5 μCi/mL). Following incubation, cells were washed (30 sec, 5 times) in ice-cold HEPES-buffered medium (as described above) to remove excess extracellular $^{22}Na^+$. Coverslips were then placed into counting vials with 1 mL of Lowry's base solution (0.4% NaOH and 2% Na_2CO_3). $^{22}Na^+$ content was determined using a Wallac Wizard 1480 (3 in. NaI crystal) gamma counter. Protein content was estimated using the method of Lowry *et al.* [20].

Normal (control) incubations were conducted in balanced electrolyte medium. Other incubations included combinations of the following drugs and treatments: (i) ouabain (10^{-2} M), a specific inhibitor of the sodium pump, was used to remove the effect of the sodium pump, (ii) monensin (40 μg/mL), a sodium ionophore, was used to artificially increase sodium entry, (iii) hypotonic medium (100 mOsM, i.e. balanced electrolyte medium at one-third strength) was used to damage cells osmotically, (iv) deoxycholate (1.2 mM), a detergent, was introduced as a chaotic agent, and (v) CPZ, an antipsychotic drug, was used routinely at 5 mM. Incubations were carried out at 37° for both rat and toad liver cells. The amphibian species used is a thermotolerant species where 37° can occur in its natural environment. These animals can be maintained for several months at this temperature in captivity.

Incubations were carried out over a duration of 20 min. This period was determined to provide the most reliable measurements of $^{22}Na^+$ entry as it maximizes the linear entry interval. In a number of preparations, liver cells were loaded with $^{22}Na^+$ by preincubating the cells for 20 min in ouabain and $^{22}Na^+$. This was followed by a 20-min treatment period (without $^{22}Na^+$) in which the cells were incubated in the presence or absence of both CPZ and ouabain. This experiment was to determine the effect of CPZ on sodium efflux.

Measurement of Liver Cell Sodium Content

Total sodium content of liver cells was measured using a Corning 410 Flame Photometer. Cells were incubated as previously described but washed (5 × 30 sec) in 100 mM (isotonic) $MgCl_2$, pH 7.4 (Tris base).

[^{3}H]Ouabain Binding

The number of [^{3}H]ouabain binding sites was determined for *R. norvegicus* liver as previously described [21]. The saturating ouabain concentration used was 5×10^{-6} M. Nonspecific sites were corrected for by subtracting ^{3}H-activity of samples incubated in excess unlabelled ouabain (10^{-2} M). Liver incubations were performed in the presence of 1 mM veratrine (as previously described in Ref. 21, veratrine is a potent inhibitor of the steroid carrier system

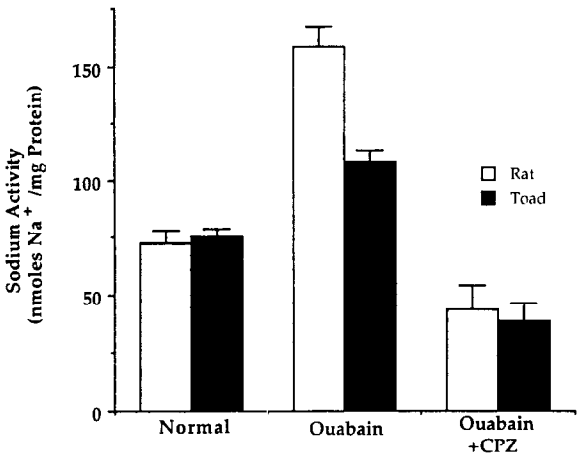

FIG. 1. Effect of 5 mM CPZ on sodium activity of isolated rat and toad liver cells. These measurements were made by incubating liver cells in balanced electrolyte medium containing $^{22}Na^+$ over a period of 20 min. In both species, the presence of ouabain at 10 mM resulted in a significant increase ($P < 0.0001$) in sodium activity within the cell. The presence of CPZ resulted in a significant reduction in intracellular sodium activity (i.e. compared with ouabain, $P < 0.0001$, and compared with normal, $P < 0.03$ to 0.0001). Values are means ± SEM; N > 21 rats and 18 toads.

present in mammalian liver cell membranes yet does not affect ouabain binding to the sodium pump [22]).

Statistics and Ethics Approval

All values are expressed as means ± SEM. Differences in sodium content of liver cells were compared using paired and unpaired *t*-tests where appropriate. All animal experiments were approved by the University of Wollongong Animal Ethics Committee.

RESULTS

To characterize the preparation, initial experiments were carried out using both rat and toad liver cells. These experiments examined the level of radioactive sodium ($^{22}Na^+$) present in liver cells after 20 min of incubation in normal medium (balanced electrolyte medium with $^{22}Na^+$), ouabain, and ouabain plus CPZ. Figure 1 shows the results of these initial experiments. Incubation in 10 mM ouabain (i.e. specific sodium pump inhibitor) showed a significant increase in the $^{22}Na^+$ content of liver cells ($P < 0.0001$). Therefore, the difference in sodium entry between normal and ouabain incubations represents $^{22}Na^+$ that would normally be removed by the activity of the sodium pump.

Incubations in the presence of CPZ plus ouabain resulted in a decrease in $^{22}Na^+$ levels. $^{22}Na^+$ levels in cells incubated with CPZ plus ouabain were significantly less than sodium levels in both ouabain and normal incubations ($P < 0.0001$ and $P < 0.03$, respectively). In accord with available literature [4, 16, 17], we initially interpreted these results to indicate that CPZ was blocking sodium entry through sodium channels.

To confirm these results, we conducted control experiments where the cells were preloaded with $^{22}Na^+$. The sodium loading involved preincubating the cells in ouabain and $^{22}Na^+$. Then these cells were incubated in medium either with or without CPZ/ouabain but with no radioactive sodium, to determine the effects of CPZ on sodium efflux. The expected outcome of this experiment was that if CPZ was reducing sodium entry, via sodium channel blockade or other sodium entry pathways, then incubating these preloaded cells in CPZ would result in the retention of their $^{22}Na^+$ load better than in cells incubated in the absence of CPZ. This outcome is based on the assumption that sodium channel blockade would reduce the dilution of the $^{22}Na^+$ with normal sodium. Therefore, $^{22}Na^+$ would be retained in the CPZ-treated cells. The results of these experiments are shown in Fig. 2 (panel A, rat; panel B, toad).

Panels A and B of Fig. 2 show that cells from both animals loaded up with $^{22}Na^+$ during the 20-min preincubation period (ouabain-loaded, compare with values in Fig. 1). When the loaded cells were incubated subsequently in ouabain, they retained 46% (in both species) of their $^{22}Na^+$. The lost sodium presumably is due to the normal diffusion processes taking place across the cell membrane. In contrast, cells incubated in ouabain and CPZ retained only 3 and 12% of their total $^{22}Na^+$ load in rat and toad, respectively. This was not the result predicted.

Results from this experiment suggest that rather than preventing sodium from entering into the liver cells, CPZ causes sodium to be removed from within the cell. To further examine this, experiments were conducted to determine the extent to which CPZ could remove sodium from within the cells. Incubations were performed in the presence of monensin, a sodium ionophore, plus ouabain, and the results are shown in Fig. 3. These results suggest (i) that monensin plus ouabain significantly increased the sodium entry over incubation in ouabain alone (compare with values in Fig. 1), demonstrating the sodium ionophore function of monensin; and (ii) that the presence of CPZ resulted in cell sodium ($^{22}Na^+$) levels being less than those of normal incubations ($P < 0.002$ for rat and 0.0003 for toad).

Two possible explanations that may account for these results involve (i) the stimulation of active sodium transport (i.e. against the sodium concentration gradient), and (ii) an increase in the sodium:sodium exchange across the cell membrane (i.e. exchange of $^{22}Na^+$ for Na^+). This latter possibility would produce the appearance of sodium moving out of the cell against its concentration gradient as radioactive sodium is exchanged for normal sodium. To discriminate between these two possibilities, the absolute intracellular levels of sodium were determined using flame photometry. These experiments were conducted on toad liver cells. The results of these experiments are shown in Fig. 4. To maximize sodium entry and elicit the greatest

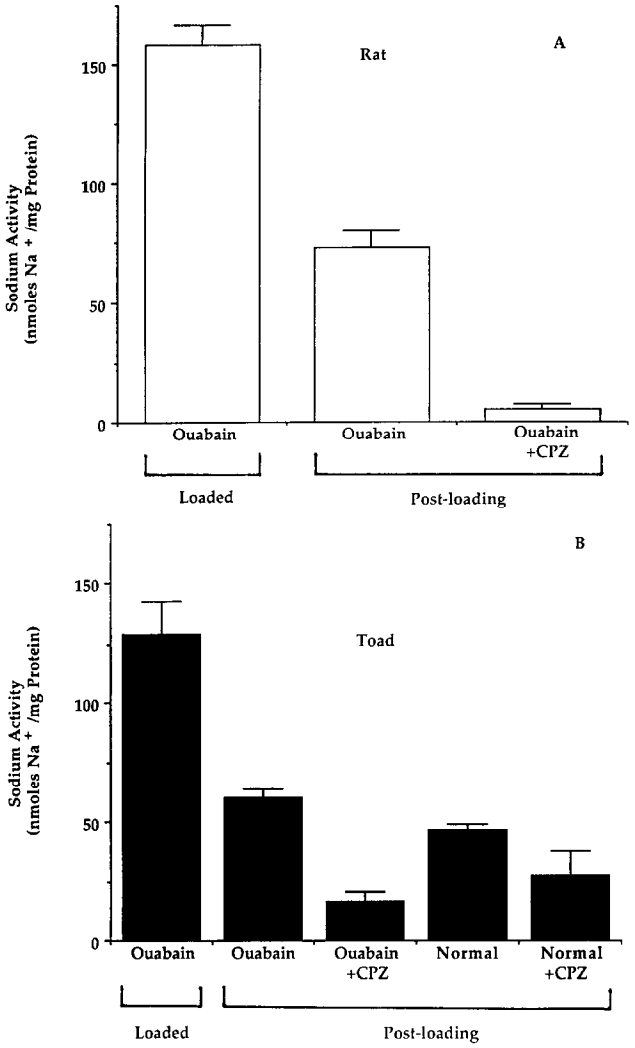

FIG. 2. Effects of CPZ on liver cells preloaded with $^{22}Na^+$ (i.e. Loaded). Loading occurred by incubating liver cells in a medium containing $^{22}Na^+$ and ouabain (10 mM) for 20 min. These cells were then placed into a variety of secondary incubations (i.e. Post-loading); normal (no drugs), normal + CPZ (5 mM), ouabain (10 mM), ouabain + CPZ. Post-loading incubations lacked $^{22}Na^+$ and lasted 20 min. All matched incubations were significantly different from one another ($P < 0.02$ to 0.0001). In Fig. 2B, normal versus normal + CPZ and ouabain versus ouabain + CPZ incubations all showed significant differences ($P < 0.001$), demonstrating that CPZ can reduce resting sodium levels. Values are means ± SEM; N = 5 for rats and toads.

CPZ effect, monensin was included in the incubations. This experiment clearly demonstrated the ability of CPZ to lower the absolute level of intracellular sodium. These results suggest that CPZ is stimulating active sodium transport rather than increasing Na^+:Na^+ exchange.

An alternative explanation for the sodium-lowering effect of CPZ is that the high concentration of CPZ required may cause damage to the cells. The relationship between CPZ concentration and intracellular sodium was examined, and activation of sodium transport was found to be dependent on the concentration of CPZ; the results are shown in Fig. 5. To examine the effect of cell damage on

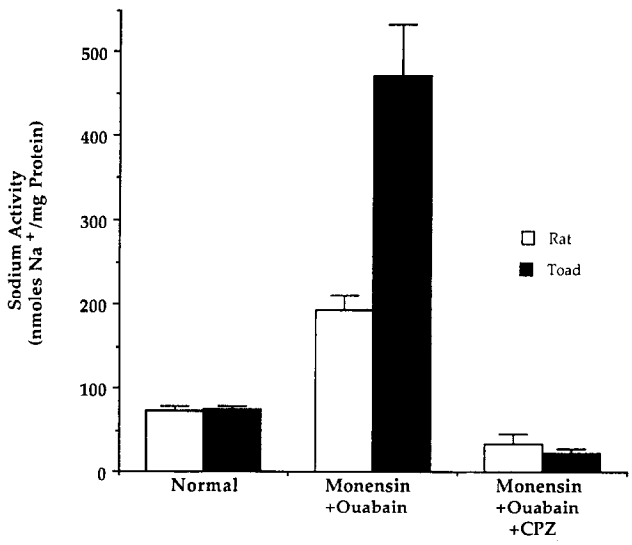

FIG. 3. Comparison of sodium activity of normal cells with those incubated with monensin (40 μg/mL) + ouabain (10 mM) and monensin, ouabain, and CPZ (5 mM). The presence of CPZ completely removed the effects of monensin and ouabain ($P <$ 0.007 to 0.0001) and reduced the sodium activity within the cells to below those of normal incubations ($P < 0.002$ for rat and 0.0003 for toad). All cells were incubated in $^{22}Na^+$ for 20 min. Values are means ± SEM; N > 7 for rats and 6 toads.

our method of measuring intracellular sodium activity, cells were damaged deliberately using two treatments: (i) osmotic assault (i.e. incubation for 20 min in 100 mOsM medium), and (ii) incubation in a detergent (deoxycholate at 1.2 mM). Results from these experiments are shown in Fig. 6. The response of the cells to both damaging treatments was to increase their $^{22}Na^+$ activity rather than to

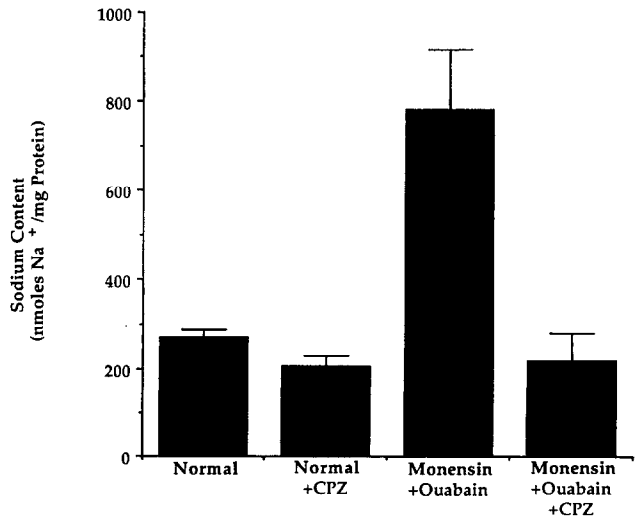

FIG. 4. Effects of CPZ on the sodium content of toad liver cells. This figure examines the sodium (Na^+) content of toad liver cells after 20 min in normal, normal plus CPZ (5 mM), monensin (40 μg/mL) plus ouabain (10 mM), and monensin, ouabain, and CPZ. All matched incubations showed significant differences ($P < 0.0001$). Values are means ± SEM; N = 5 for all treatments.

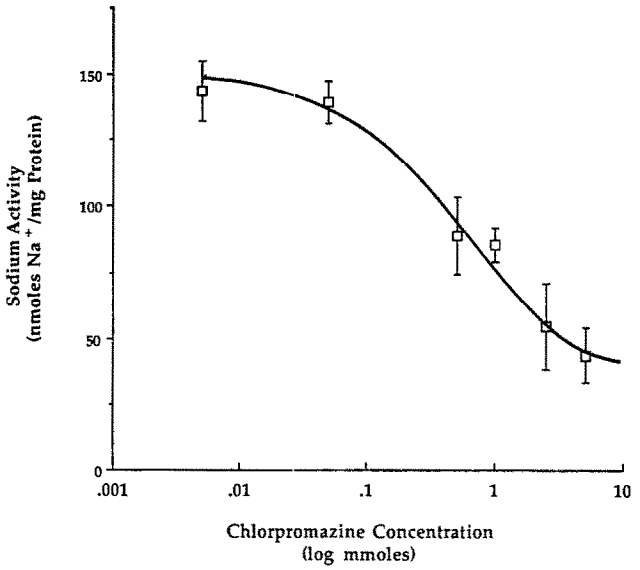

FIG. 5. Effects of CPZ concentration on cell sodium activity, measured using $^{22}Na^{+}$, in the presence of 10 mM ouabain. Values are means ± SEM; N > 4.

decrease it, as found for CPZ. These results suggest that cell damage does not account for the CPZ-linked sodium-lowering effect.

Since one of the obvious potential sites for activation of sodium transport is the sodium pump, the ability of CPZ to bind to the extracellular ouabain binding site was investigated. Our experiments showed that for rat liver the number of ouabain binding sites was 721 ± 141 pmol/g, but in the presence of CPZ (5 mM) the concentration of ouabain binding sites fell 90% to 75 ± 33 pmol/g of tissue (N = 5), indicating that CPZ has an antagonistic effect on ouabain binding.

All incubations discussed thus far have included ouabain.

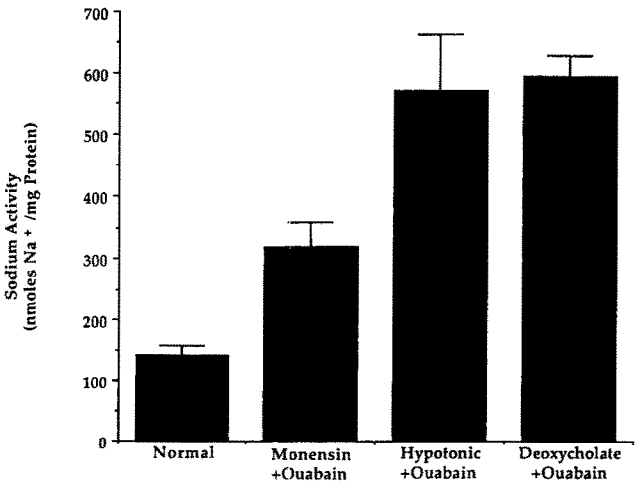

FIG. 6. Effects of cell damage on sodium activity. Toad liver cells were damaged artificially by hypo-osmotic (100 mOsM) and detergent (deoxycholate at 1.2 mM) treatments, and sodium ($^{22}Na^{+}$) activity was measured. Values are means ± SEM; N = 5.

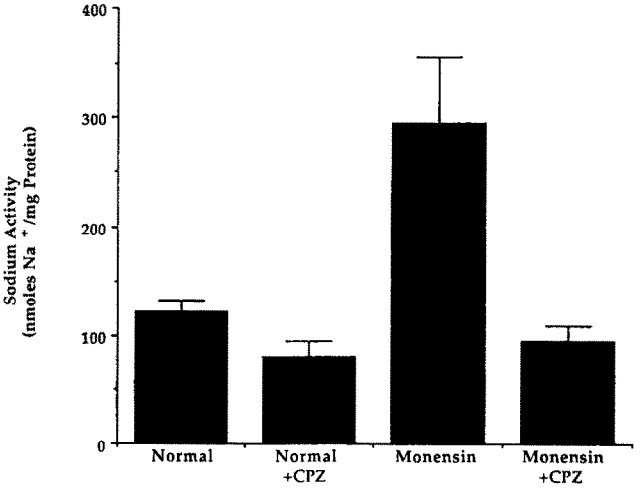

FIG. 7. Effects of CPZ at 5 mM on toad liver cell sodium activity in the absence of ouabain. The presence of CPZ resulted in significant decreases in intracellular $^{22}Na^{+}$ activity ($P < 0.0001$) in both normal and monensin (40 μg/mL) incubations. Values are means ± SEM; N = 5.

It is possible that the apparent sodium-lowering effect of CPZ could be due to CPZ antagonism of ouabain binding, thereby allowing the sodium pump to continue to operate in the presence of ouabain. To test this possibility, incubations were carried out using CPZ on normal and monensin incubations in the absence of ouabain. The results of these experiments are shown in Fig. 7. In both normal and monensin incubations, the presence of CPZ, in the absence of ouabain, produced a significant sodium-lowering effect ($P < 0.0001$ for both normal versus normal + CPZ and monensin versus monensin + CPZ). These results are also supported by a post-loading experiment (shown in Fig. 2B) where normal cells in the presence of CPZ (i.e. absence of ouabain) lowered their sodium levels below that of normal cells without CPZ.

DISCUSSION

The experimental results of this study suggest that CPZ, at high concentration, reduces intracellular sodium content. Furthermore, the decrease in intracellular sodium is not brought about by a reduction in sodium influx but by activation of an outwardly directed sodium transport mechanism. The sodium-lowering effect of CPZ was shown initially by incubating liver cells in the presence of ouabain, a sodium pump inhibitor. Under normal conditions, ouabain significantly increases intracellular sodium levels. However, in the presence of CPZ and ouabain, a significant reduction in the sodium levels occurred. This reduction, brought about by CPZ, resulted in the sodium levels of treated cells being less than that of normally incubated cells. These results were secondarily confirmed by experiments showing that CPZ alone would significantly lower intracellular sodium levels. This sodium-lowering effect was first measured using $^{22}Na^{+}$ flux (Figs. 1 and 7) and later confirmed using total sodium measurements (Fig. 4).

Demonstration that the sodium-lowering effect of CPZ was due to activation of an outwardly directed sodium transport mechanism was shown using cells preloaded with $^{22}Na^+$. Incubation of sodium-loaded cells in CPZ and ouabain resulted in only 3 and 12% of the total preloaded $^{22}Na^+$ (rat and toad, respectively) being retained versus 46% retention in cells treated with ouabain alone (see Fig. 2). Therefore, the cells incubated in CPZ showed a high sodium turnover resulting in a lower sodium content. Since the movement of sodium was against the natural concentration gradient, an active rather than a passive transport mechanism is suggested.

To further prove that CPZ activated sodium transport, cells were incubated in ouabain plus monensin, a sodium ionophore. This incubation artificially induces a rapid rise in intracellular sodium levels through the monensin pathway and sodium pump inhibition. Monensin even in the absence of ouabain also caused a dramatic rise in intracellular sodium levels (compare Figs. 3 and 7 for $^{22}Na^+$) as it overcame the ability of the sodium pump to respond. The ability of CPZ to produce high sodium turnover (against monensin) was measured using both total sodium and $^{22}Na^+$ content (Figs. 3, 4, and 7). In all treatments (i.e. normal, monensin, and monensin + ouabain), the presence of CPZ resulted in reduced intracellular sodium measured in $^{22}Na^+$ or total sodium. Comparing Fig. 4 with Fig. 7, it can be seen that similar treatments but different measurement methods (i.e. $^{22}Na^+$ or total sodium) produced the same results (regardless of the presence of ouabain in the monensin incubations, i.e. Fig. 4). This indicated a considerable potential for the stimulation of sodium transport by CPZ. Therefore, these experiments demonstrated that (i) CPZ stimulates sodium transport (measured by both $^{22}Na^+$ and total sodium content), and (ii) the CPZ-activated sodium transport mechanism has a substantial capacity.

Activation of sodium transport is a property previously unreported for this drug. Previous investigations into the relationship between CPZ and sodium transport have centered around the sodium pump (i.e. Na^+, K^+-ATPase). This work has shown that CPZ inhibits rather than activates the sodium pump. This work comes from Na^+, K^+-ATPase assays carried out for liver [6, 23] and other tissues [24]. A possible explanation for this discrepancy is that Na^+, K^+-ATPase assays are normally determined as the ouabain-inhibitable portion of a Mg^{2+}-ATPase assay. If CPZ competed with or prevented ouabain from binding to the sodium pump, no Na^+, K^+-ATPase activity would be measured. This may lead to the false impression of CPZ inhibition. Since activation of sodium transport by CPZ suggests a possible interaction with a sodium transport site, CPZ may bind to or interact with the ouabain binding to the sodium pump.

An interaction between CPZ and the sodium pump was demonstrated in a [^{3}H]ouabain binding assay where CPZ (5 mM) decreased ouabain binding by 90% in intact liver. However, when we performed an Na^+, K^+-ATPase assay (using kidney homogenate due to its high intrinsic activity according to the assay method described in Ref. 21; results not shown) using both ouabain and K^+-free assays, we confirmed previous reports showing that CPZ inhibited Na^+, K^+-ATPase activity. Therefore, *in vitro* inhibition of the Na^+, K^+-ATPase activity remains in opposition to the activation of sodium transport *in vivo* (i.e. intact cells).

A well-described interaction of CPZ is its ability to block voltage-sensitive sodium channels [16, 17]. Our results identify an alternative possible explanation for some of this apparent blockade. The results of the present study suggest that CPZ-stimulated sodium transport begins at about 10 μM in liver (see Fig. 5) and is within the range of concentrations commonly used in these experiments (i.e. 10–100 μM). Therefore, CPZ-stimulated sodium transport may contribute to some of the apparent sodium channel inhibition. CPZ is also known to inhibit calcium channels and the Ca^{2+}/Mg^{2+}-ATPase enzyme [7–9, 25]. A further possibility is that just as CPZ treatment gives the initial impression of sodium channel inhibition, it might also produce the same impression on calcium ion movement, i.e. calcium channel inhibition.

It has been proposed that increased intracellular Na^+ levels could be a means of controlling the release of calcium from intracellular stores. The binding of this Ca^{2+} with calmodulin could then control various physiological processes, such as neurotransmitter release [26]. CPZ is recognized as one of the most effective inhibitors of calmodulin [27], and, therefore, it is possible that the effect of CPZ on calmodulin may alternatively be controlled through intracellular sodium lowering.

One aspect of the CPZ effect is the high concentration required to produce the activated sodium transport. A known effect of CPZ at high concentrations is its ability to cause cell damage [28, 29]. When cell damage was induced artificially via hypo-osmotic and detergent treatments, sodium levels of cells rose dramatically in contrast to the CPZ effect (see Fig. 6). This suggests that the CPZ effect is not simply related to a cell damage phenomenon. The effect of CPZ on lowering intracellular sodium is concentration dependent, beginning at approximately 10 μM (using the $^{22}Na^+$ flux method, Fig. 5) but not peaking until in the millimolar range. This may partially explain why other studies using isolated liver cells at lower CPZ concentrations may not have shown the same effect (e.g. Van Dyke and Scharschmidt [6] at 300 μM, investigating liver cholestasis caused by CPZ).

In conclusion, the phenothiazine CPZ appears not to cause weight gain through a decrease in passive permeability of cell membranes to ions such as sodium and a concomitant reduction in active transport. In contrast, an examination of sodium ion movement across liver cell membranes (using both $^{22}Na^+$ and total sodium) suggests that CPZ has an ability to reduce intracellular sodium content. The reduction in sodium content appears to be due to activation of an outwardly directed (against the sodium concentration gradient) sodium transport mechanism. This effect occurred in the presence or absence of the sodium pump

inhibitor ouabain, and additionally in the presence of the sodium ionophore monensin. Although the concentration used was nonpharmacological, further investigation of similar compounds combined with computer modelling may produce a drug active at lower concentration. Our future work will also be aimed at determining the mechanistic basis of this effect. The benefits of such a drug may lie in the ability to control sodium ion levels during hypoxia or in areas related to active transport, such as renal and cardiovascular pathologies.

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