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EFFECT OF CHLORPROMAZINE ON PERMEABILITY OF THE TOAD BLADDER*

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Abstract—In concentrations between 2×10^{-5} and 2×10^{-4}M, chlorpromazine stimulates active sodium transport by the urinary bladder of the toad, *Bufo marinus*. The rise in sodium transport is associated with an increase in oxygen consumption and a fall in direct current electrical resistance. Chlorpromazine impairs vasopressin-induced osmotic water flow across the membrane; this impairment is associated with an irreversible increased permeability to solute. It is postulated that chlorpromazine alters membrane permeability. When osmotic water flow is subsequently induced, the integrity of the drug-treated cell membranes is grossly disrupted.

THERE has been much recent evidence that chlorpromazine alters the permeability of biological membranes. This evidence has come from studies of protozoa,[1] skeletal muscle,[2] erythrocytes,[3] mitochondria[4] and other membranous particles.[5] It has been proposed that changes in permeability are a basic effect of the phenothiazines and may explain many of the actions of these drugs.[5]

The urinary bladder of the toad has been extensively used as a model system for studying water and sodium permeability and transport.[6] This tissue actively transports sodium ion from its mucosal (urinary) to its serosal (peritoneal) surface. In addition, it greatly increases its osmotic permeability to water in the presence of vasopressin. The modification of these processes by chlorpromazine is the subject of this report.

EXPERIMENTAL

All experiments were carried out with the urinary bladder of the toad (*Bufo marinus*). For electrical measurements, paired hemibladders were mounted between halves of lucite chambers and bathed on both mucosal and serosal surfaces by 20 ml of frog-Ringer's solution. Chlorpromazine HCl precipitated when added to Ringer's solution of pH 7·8 to 8·0, the pH most commonly employed in studies of the toad bladder, whereas it dissolved completely in solutions of pH 7·1 and 6·3. A solution of the latter

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pH had been used satisfactorily in this laboratory in the past[7,8] and hence was employed in the present studies: Na 111, K 5·0, Ca 1·8, Cl 114, HPO_4 2·0, and H_2PO_4 2·0 mequiv./l., adjusted to pH 6·3 with HCl; 220 mosmoles/kg H_2O. In certain experiments the mucosal bathing medium was sodium-free choline Ringer's, in which all sodium was replaced by choline ion. The potential difference and short-circuit current were monitored according to the method of Ussing and Zerahn.[9] The direct current (d.c.) resistance was calculated as the change in potential difference produced by a given change in current. Short-circuit current and d.c. resistance are expressed per 4·5 cm^2 of exposed membrane. Ordinarily no variables were imposed for several hours after the hemibladders were mounted. Chlorpromazine was then added to one of the hemibladders, the other half serving as a control. The time of addition was designated time zero. Subsequent values were normalized by expressing them as a per cent of the value at time zero.

Oxygen consumption was measured by manometry. Two bladders were cut into sixteen approximately equal segments. These were gently blotted and distributed at random in four Warburg vessels, each containing 2·4 ml of Ringer's solution. The center well contained a strip of filter paper dipped in 0·2 ml of 20% KOH. Two of these vessels served as controls and contained 0·1 ml of Ringer's solution in the sidearms. The sidearms in the remaining two vessels contained chlorpromazine dissolved in 0·1 ml of Ringer's solution. The concentration of chlorpromazine in the sidearms was such that a final concentration of either 10^{-3} or 10^{-4} M was achieved when the contents of the sidearms were added to the main pool. An additional two manometers, containing the incubation solutions but no tissue, served as thermo-barometers. All experiments were carried out at 27°. Measurements were made at three consecutive 40-min intervals. The contents of the sidearm were added at the end of the first 40-min period. The oxygen consumption values were normalized by expressing the oxygen consumption in the second and third 40-min periods as a percentage of the oxygen consumption in the first 40-min period. Statistical significance was evaluated by the Wilcoxon rank test.

Water flow was measured with the hanging bag technique described by Bentley.[10] Paired hemibladders were mounted as bags with the urinary surface forming the inner lining of the bag. Each bag was filled with 5 ml of distilled water and suspended in a 50-ml bath of Ringer's solution of the same composition described above, except that in specified experiments the pH was brought to pH 7·1. After a period of equilibration, vasopressin or chlorpromazine or both were added to the serosal bath of appropriate bags and this time was designated time zero. At 10-min intervals the bags were gently blotted to remove excess water and then weighed. From the measured weight loss of each bag, the rate of transepithelial water movement was assessed. At 15-min intervals a 200-μl aliquot was removed from each bag and its osmolality determined (Osmometer model no. G62, Fiske Associates, Bethel, Conn.). The 200 μl was immediately replaced with 200 μl of distilled water. From the weight of the fluid remaining in the bag and its osmolality, the total amount of solute in the bag (as microosmoles) was calculated. Experiments were continued for 60–120 min, at which time the Na^+ and K^+ concentration of the fluid remaining in the bag was determined by flame photometry (model 143, Instrumentation Laboratory Inc., Watertown, Mass.). Chlorpromazine HCl was obtained as a gift from Dr. Irene Forrest. Vasopressin was used as Pitressin (Parke, Davis & Company, Detroit, Mich.).

RESULTS

Effect of chlorpromazine on electrical characteristics

In the presence of active sodium transport. The effect of chlorpromazine (CPZ), 7 to 10 $\times$ 10^{-5}M, on the potential difference, short-circuit current (SCC) and d.c. resistance of 10 hemibladders bathed in sodium Ringer's solution is presented in Table 1. The potential difference rose by 17 per cent at 30 min after addition; effects thereafter were variable and statistically insignificant, although the tendency was a decline.

TABLE 1. EFFECT OF CHLORPROMAZINE ON ELECTRICAL CHARACTERISTICS OF TOAD BLADDER IN THE PRESENCE OF SODIUM TRANSPORT (N = 10)

Electrical characteristics		Value at time/value at $time_0$ $\times$ 100			
		30 min	60 min	90 min	120 min
Potential difference (mV)					
CPZ	50	119·6	113·7	85·4	61·3
Control	43	103·1	99·1	92·5	84·7
Difference	7	+16·5	+14·6	−7·1	−23·4
S.E.	±7	±6.1	±7·3	±9·1	±11·8
P	NS	<0·05	NS	NS	NS
Short-circuit current (μamp)					
CPZ	138	134·6	139·6	118·2	103·4
Control	139	100·5	95·0	88·8	81·1
Difference	1	+34·1	+44·6	+29·4	+22·3
S.E.	±17	±9·9	±11·4	±14·2	±20·3
P	NS	<0·01	<0·01	NS	NS
Direct-current resistance (Ω)					
CPZ	387	90·5	84·2	78·5	62·4
Control	311	104·1	103·0	105·6	107·1
Difference	76	−13·6	−18·8	−27·1	−44·7
S.E.	±50	±4·6	±6·4	±9·8	±6·4
P	NS	<0·02	<0·02	<0·05	<0·01

* The concentration of chlorpromazine employed was 7–10 $\times$ 10^{-5}M in the serosal bathing medium. Standard errors are calculated from the ten individual paired differences. CPZ = chlorpromazine; NS = not significant.

The effects on the short-circuit current are illustrated in Fig. 1. The SCC rose by 34 per cent at 30 min and by 45 per cent at 60 min; during the next hour, the elevations above the control levels were variable and not statistically significant.

The d.c. resistance declined steadily and significantly after the addition of CPZ; in 2 hr it had fallen by 45 per cent. In additional experiments, a rise in SCC was obtained with concentrations of CPZ as low as 2 $\times$ 10^{-5}M. CPZ at 10^{-3}M regularly inhibited the SCC. The drug was equally effective added to the mucosal (urinary) or serosal bathing medium.

In the absence of active sodium transport. Active sodium transport by the toad bladder can be abolished by replacing sodium ion with choline ion in the mucosal bathing medium. The effect of such a change is shown in Table 2. The transmembrane potential difference, and hence the SCC, is abolished or sometimes even slightly reversed. The d.c. resistance rises substantially. Over the ensuing hour, the potential difference and SCC rise slightly but significantly, presumably due to a rising concentration of sodium in the mucosal bath through diffusion from the serosal bath.

To evaluate the effect of CPZ on the bladder in the absence of sodium transport, sodium was replaced with choline in the mucosal medium of five hemibladders while

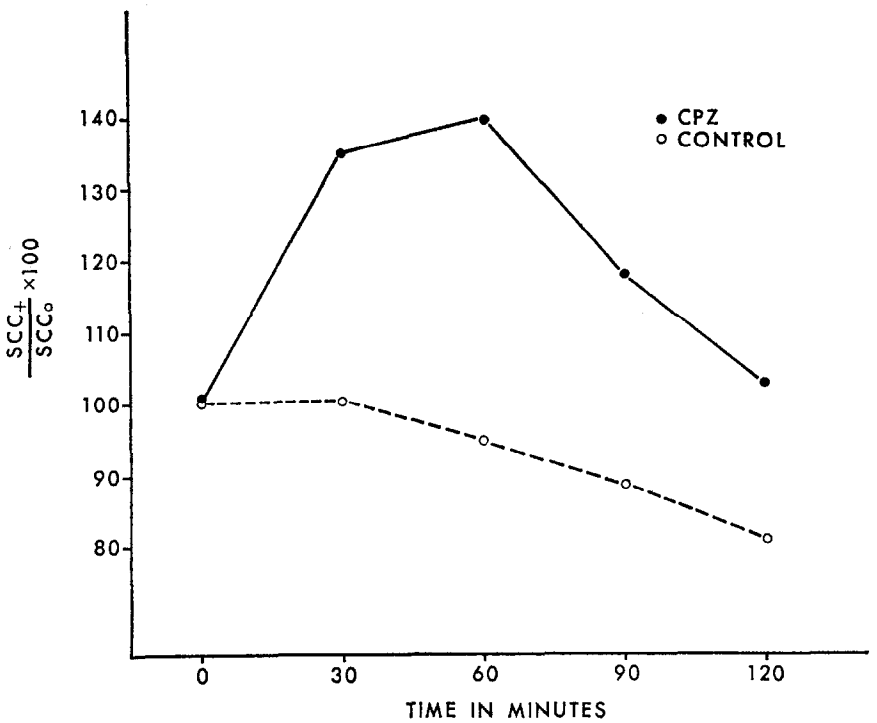

FIG. 1. Stimulation of the short circuit current by chlorpromazine. Chlorpromazine was added at a concentration of 7 to 10 $\times$ 10^{-5}M at time zero to ten hemibladders. Control paired hemibladders received no addition. Differences at 30 and 60 min were statistically significant ($P < 0{\cdot}01$); differences at 90 and 120 min were not significant.

their control hemibladders remained in sodium Ringer's. CPZ, 10^{-4}M, was added to each hemibladder 30 min after the mucosal fluid change. As seen in Table 3, the sodium-transporting hemibladders responded in a manner similar to that reported in Table 1. However, the non-sodium-transporting hemibladders demonstrated minimal changes in potential difference and SCC, and these changes were comparable to those seen in the absence of CPZ (Table 2). In contrast, the d.c. resistance dropped substantially and progressively after addition of CPZ to both the transporting and non-transporting hemibladders; the absolute fall of both groups was the same.

In the presence of vasopressin. Vasopressin stimulates active sodium transport by the toad bladder.[11] To determine the effect of CPZ on this stimulation, one hemibladder of a pair received vasopressin, 50 mU/ml, while the other was simultaneously challenged with this amount of vasopressin plus CPZ, 10^{-4}M. All additions were made serosally. In seven experiments, the maximum increase in SCC averaged 33 $\pm$ 9 per cent greater in the hemibladders receiving CPZ.

Effect of chlorpromazine on oxygen consumption

The effect of different concentrations of CPZ on oxygen consumption is illustrated in Fig. 2. CPZ, 10^{-4}M, increased the oxygen consumption of bladders in two consecutive 40-min periods ($P < 0{\cdot}02$). In contrast, CPZ, 10^{-3}M, inhibited oxygen consumption ($P < 0{\cdot}02$).

TABLE 2. EFFECT ON ELECTRICAL CHARACTERISTICS OF TOAD BLADDER WHEN MUCOSAL FLUID IS CHANGED FROM SODIUM RINGER'S TO CHOLINE RINGER'S (N = 6)

Electrical characteristics	Pre-choline	Post-choline (min)			Increment from 30 to 90 min
		30	60	90	
Potential difference (mV)	46 ± 4	0 ± 1	3 ± 1	4 ± 1	+4 ± 1
Short-circuit current (μamp)	304 ± 69	2 ± 2	9 ± 3	11 ± 4	+9 ± 3
Direct current resistance (Ω)	147 ± 23	347 ± 29	357 ± 14	358 ± 26	+11 ± 11

TABLE 3. EFFECT OF CHLORPROMAZINE ON ELECTRICAL CHARACTERISTICS OF TOAD BLADDER IN THE ABSENCE OF SODIUM TRANSPORT (N = 5)

Electrical characteristics	Time after addition of chlorpromazine (min)					Change from 0 to 60 min
	−30 min*	0	30	60	90	
Potential difference (mV)						
A	72	61	63	49	29	−12 ± 7
B	68	−6	−3	−2	0	+5 ± 2
Difference	4	67	66	51	29	−17
S.E.	±7	±6	±5	±6	±5	±7
Short-circuit current (μamp)						
A	277	206	268	278	220	+72 ± 25
B	274	−10	−6	−3	−1	+7 ± 4
Difference	3	216	274	281	221	+65†
S.E.	±55	±25	±30	±36	±46	±25
Direct-current resistance (Ω)						
A	271	311	241	179	139	−132 ± 19
B	282	632	543	490	442	−140 ± 36
Difference	11	321	302	311	303	+8
S.E.	±45	±55	±52	±48	±39	±19

* All hemibladders bathed in sodium Ringer's. Immediately thereafter, the mucosal fluid of hemibladder B was changed to choline Ringer's and remained so throughout.
† $P < 0.1$. At 30 min, the difference was +58 ± 10 ($P < 0.01$).

Effect of chlorpromazine on osmotic water flow and solute permeability

In the absence of vasopressin. In Table 4 are shown the results of eight paired experiments in which CPZ, 7×10^{-5}M, was added to the serosal bathing medium of one hemibladder bag and an equal volume of diluent only was added to the other bag. There was no significant effect of the drug on water loss from the bag in any 30-min period. Over the 2-hr period, however, there was a significant ($P < 0.05$) but slight increase in water loss. There was a comparably small but steady accumulation of solute in both the experimental and control bags.

In the presence of vasopressin. In the presence of an osmotic gradient, vasopressin induces a net water flow across the toad bladder. The effect of CPZ on this water flow is documented in Table 5. Results of experiments carried out at pH 7·1 are shown in the upper half of the table and are illustrated in Fig. 3.

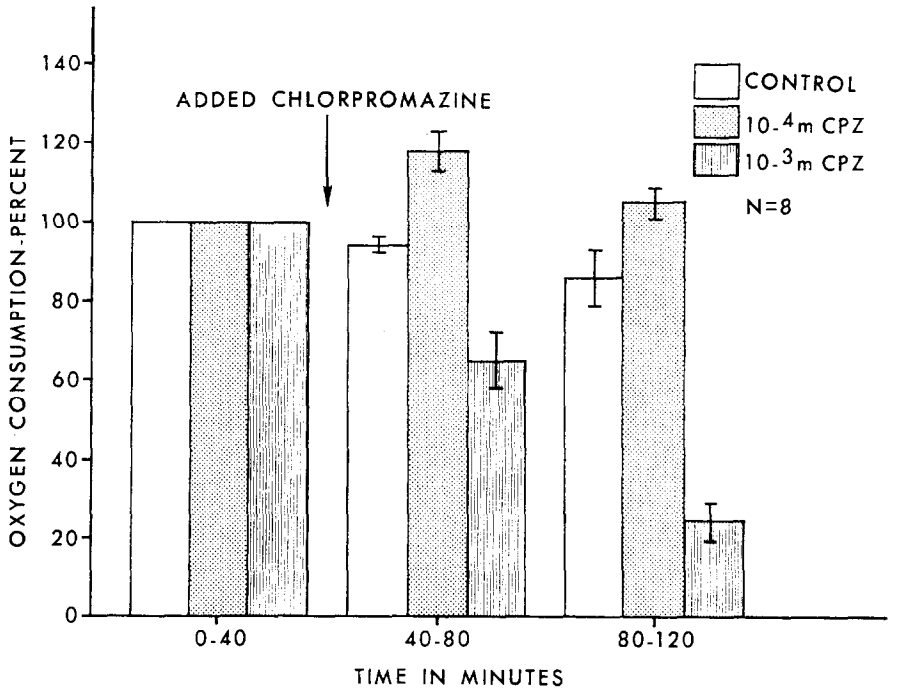

FIG. 2. Effect of chlorpromazine on oxygen consumption. Results are normalized using the 0–40 min control period as 100 per cent. The vertical bars denote 1 S.E. of the mean. At 40–80 and 80–120 min, CPZ, 10^{-4}M, stimulated oxygen consumption ($P < 0{\cdot}02$), whereas CPZ, 10^{-3}M, inhibited it ($P < 0{\cdot}02$).

TABLE 4. EFFECT OF CHLORPROMAZINE ON OSMOTIC WATER FLOW AND SOLUTE PERMEABILITY IN THE ABSENCE OF VASOPRESSIN (pH 6·3; N = 8)

	0–30 min	30–60 min	60–90 min	90–120 min	0–120 min
Weight loss (g)					
CPZ (7×10^{-5}M)	0·22	0·14	0·11	0·13	0·60
Control	0·15	0·10	0·11	0·08	0·44
Difference	+0·07	+0·04	0	+0·05	+0·16
S.E.	±0·04	±0·03	±0·03	±0·04	±0·065
P	NS	NS	NS	NS	<0·05
Solute gain (μosmoles)					
CPZ (7×10^{-5}M)	7	4	15	13	39
Control	14	0	3	6	23
Difference	−7	+4	+12	+7	+16
S.E.	±8	±8	±6	±4	±10
P	NS	NS	NS	NS	NS

TABLE 5. EFFECT OF CHLORPROMAZINE ON OSMOTIC WATER FLOW AND SOLUTE PERMEABILITY IN THE PRESENCE OF VASOPRESSIN (N = 8)*

	Weight loss (g)				Solute gain (μosmoles)			
	0–30 min	30–60 min	60–90 min	90–120 min	0–30 min	30–60 min	60–90 min	90–120 min
pH 7·1								
Vasopressin + CPZ	0·87	0·49			47	63		
Vasopressin	1·09	1·10			1	7		
Difference	−0·22	−0·61			+46	+56		
S.E.	±0·19	±0·16			±18	±10		
P	NS	<0·01			<0·05	<0·01		
pH 6·3								
Vasopressin + CPZ	0·30	0·40	0·30	0·22	35	74	94	94
Vasopressin	0·26	0·44	0·45	0·44	2	4	13	2
Difference	+0·04	−0·04	−0·15	−0·22	+33	+70	+81	+92
S.E.	±0·03	±0·03	±0·05	±0·03	±11	±12	±12	±17
P	NS	NS	<0·02	<0·01	<0·02	<0·01	<0·01	<0·01

* Vasopressin concentration, 100 mU/ml in serosal medium; chlorpromazine concentration, 7×10^{-5}M in serosal medium.

CPZ markedly attenuated the vasopressin-induced net movement of water. This effect was evident by 30 min and became progressively more pronounced. In the second 30-min period, CPZ-treated bags lost 45 per cent as much weight as their untreated controls and in the final 10 min they lost only 29 per cent as much.

An accumulation of solute was observed in bags exposed to CPZ plus vasopressin but was negligible in those exposed to vasopressin alone. The accumulation became significant ($P < 0.05$) in the second 15-min period. Analysis revealed that approximately half of the osmolality appearing in the CPZ plus vasopressin bags was contributed by sodium plus potassium in a ratio (K/Na) slightly greater (0·064 ± 0·004) than that of the Ringer's solution in which the bags were bathed (0·045). Similar effects on water and solute were obtained when CPZ was added to the mucosal bath.

To afford a better comparison with the electrical studies, which were carried out at pH 6·3, water flow experiments were repeated at this lower pH (Fig. 4). The effect of vasopressin on water flow is substantially reduced when pH is below 7.[12] Inhibition by CPZ appeared more slowly (60 min), but once established, its relative magnitude was comparable to that found at high pH, reaching 50 per cent in the final half-hour period. Although the absolute water flow at pH 6·3 was 60–80 per cent less than at pH 7·1 the rate of solute accumulation in the bags was as great as that at pH 7·1. Furthermore, the onset of solute accumulation began in the first 30-min period, clearly prior to any effect on water flow.

Cyclic 3′,5′-AMP has been shown to mimic the effects of vasopressin on the toad bladder.[13] Six paired experiments were performed at pH 7·1 using cyclic 3′,5′-AMP, 10 mM, in place of vasopressin. CPZ inhibited water flow by 29 ± 8 per cent over a 60-min period and produced an accumulation of solute which was 61 ± 13 μosmoles greater than controls receiving cylic 3′,5′-AMP alone.

A series of experiments were conducted to examine the reversibility of these drug effects. Eight paired bags were exposed to 100 mU/ml of vasopressin; one member of each pair was challenged with 7×10^{-5}M CPZ. After 75 min, all bags were washed repetitively for 30 min with fresh solution; the inside (mucosal face) of the bags was

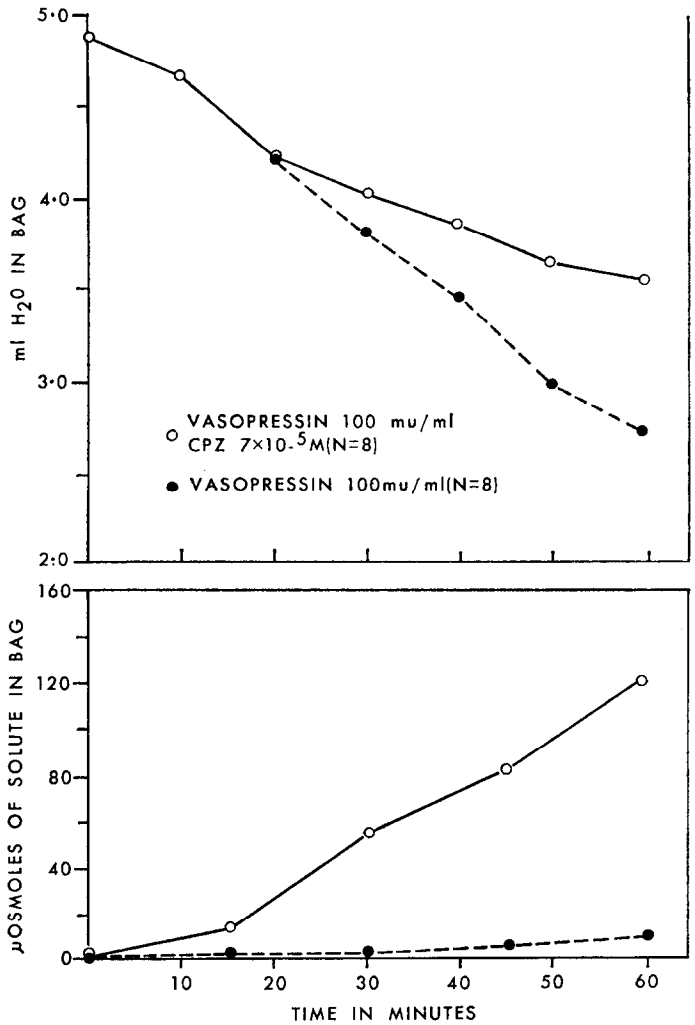

FIG. 3. Effect of chlorpromazine on osmotic water flow (above) and solute permeability (below) at pH 7·1. Initially the inside of the bag contained distilled water while the outside was bathed in sodium Ringer's solution. Additions of vasopressin and chlorpromazine were made at time zero. Differences in water flow were significant in the interval between 30 and 60 min ($P < 0{\cdot}01$); differences in solute gain were significant at 0–30 min ($P < 0{\cdot}05$) and at 30–60 min ($P < 0{\cdot}01$).

washed with distilled water and the outside (serosal face) with sodium Ringer's. The bags were then refilled with 5 ml of distilled water and placed in a bath of sodium Ringer's for a 30-min equilibration period. All bags were then challenged with 100 mU/ml of vasopressin and their weight loss was assessed over the next hour. In the first 75-min period, the characteristic dyad of solute leak and attenuated water flow was observed in bags exposed to CPZ. During the 30-min equilibration period, the CPZ-treated bags were leaky to solute while the untreated controls were not; neither bags lost weight. When rechallenged with vasopressin, the bags not previously exposed to CPZ responded with a water loss comparable to that seen originally; the bags

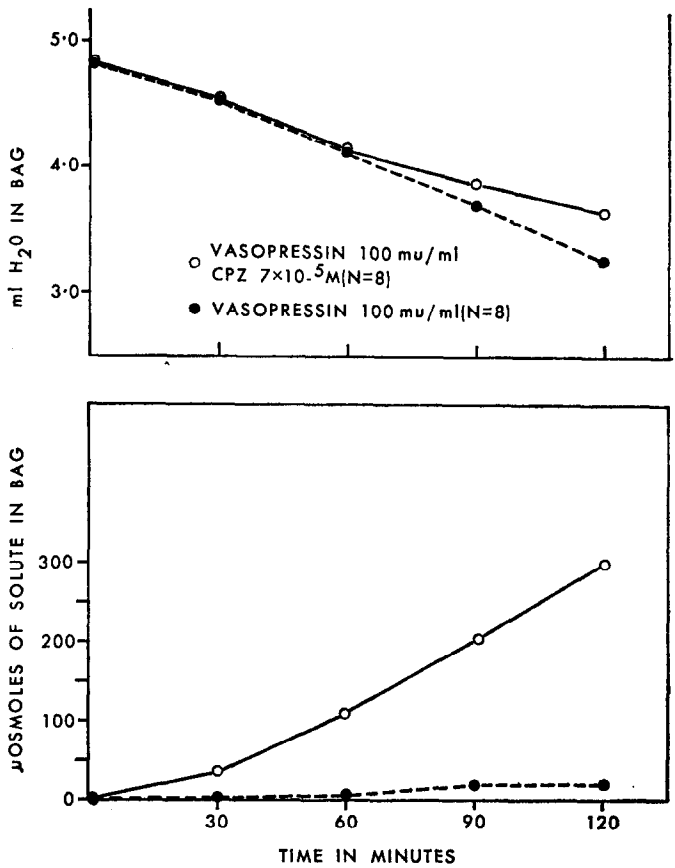

FIG. 4. Effect of chlorpromazine on osmotic water flow (above) and solute permeability (below) at pH 6·3. The protocol followed was the same as in Fig. 3. Differences in water flow were significant in the interval between 60 and 90 min ($P < 0{\cdot}02$) and between 90 and 120 min ($P < 0{\cdot}01$); differences in solute gain were significant ($P < 0{\cdot}02$) in each 30-min interval.

previously exposed to chlorpromazine lost 48 $\pm$ 15 per cent as much water as the controls and continued to accumulate solute.

To determine if this persistence of the effect of CPZ was due to failure to wash the drug out of the tissue, the serosal surface of one of each pair of eight hemibladder bags was exposed for 30 min to Ringer's solution containing CPZ, 7×10^{-5}M, while the other was exposed to Ringer's solution only (pH 7·1); the mucosal medium was distilled water. The bags were then washed repetitively for 30 min with fresh Ringer's solution externally and distilled water internally. The bags were then refilled with 5 ml of distilled water and placed in sodium Ringer's. After 30 min of equilibration, all bags were exposed to vasopressin, 100 mU/ml. Over the ensuing 90 min, water loss was identical in both CPZ-treated and untreated bags; solute accumulation did not occur. It was concluded that CPZ can be successfully washed from the tissue.

These results suggested that solute accumulation occurred when water flow was induced through the tissue in the presence of CPZ. Therefore an experiment was

carried out in which CPZ and vasopressin were present, but osmotic water flow was prevented by failure to provide an osmotic gradient. Five paired bags were filled with a solution of mannitol in distilled water, 220 mosmoles/kg H_2O, and placed in a bath of sodium Ringer's (pH 7·1). One of each pair was exposed to CPZ, 7×10^{-5}M, and all bags were challenged with vasopressin, 100 mU/ml. Solute accumulation was evaluated by measuring the concentrations of sodium and potassium in the bags.

TABLE 6. EFFECT OF CHLORPROMAZINE ON SOLUTE PERMEABILITY IN THE PRESENCE OF VASOPRESSIN BUT THE ABSENCE OF OSMOTIC WATER FLOW (pH 7·1; N = 5)

Time (min)	Weight loss (g)			Solute gain (μequiv. $Na^+ + K^+$)		
	0–30 min	30–60 min	0–60 min	0–30 min	30–60 min	0–60 min
Vasopressin + CPZ	0·20	0·04	0·24	+0·1	+1·0	+1·1
Vasopressin	0·15	0·07	0·22	+0·5	+0·8	+1·3
Difference	+0·05	−0·03	+0·02	−0·4	+0·2	−0·2
S.E.	±0·06	±0·02	±0·06	±0·4	±1·4	±1·7
P	NS	NS	NS	NS	NS	NS

The results, presented in Table 6, indicate that the absence of an osmotic gradient prevented net water movement. CPZ induced no solute accumulation over a 60-min period. When the iso-osmotic mannitol was subsequently replaced by distilled water, the characteristic effects of CPZ on water and solute (as reflected by sodium and potassium accumulation in these bags) were observed. It was concluded that osmotic water flow is required to induce solute accumulation.

DISCUSSION

The effects of chlorpromazine seen in the present study can best be interpreted in the light of contemporary concepts of the way in which sodium is actively transported by the toad bladder. This transepithelial transport is considered to take place in two steps.[6] In the first step, sodium enters the cell across its mucosal surface under the driving force of a concentration gradient. The low intracellular concentration of sodium is maintained by the "sodium pump", the second step, which actively extrudes sodium across the serosal surface. According to this theory, sodium transport may be augmented by any agent which: (1) increases the permeability of the mucosal barrier and permits a greater entry of sodium into the cell, or (2) directly stimulates the pump or increases its energy supply.

Under usual conditions, the SCC is a measure of the active transport of sodium from the urinary to the serosal surface of the toad bladder.[11] CPZ in concentrations between 2×10^{-5} and 2×10^{-4}M stimulates the SCC. When sodium in the mucosal bathing medium is replaced with choline, CPZ fails to produce this effect. This strongly suggests that the rise in SCC is a manifestation of increased transepithelial sodium transport. Since this is an active, energy-requiring process it ought to be accompanied by an increased oxygen consumption. This was shown so. At a higher concentration (10^{-3}M), CPZ inhibits both the SCC and oxygen consumption. It is noteworthy that

a biphasic response to CPZ has been observed in different tissues for a number of biochemical parameters.[5]

Evidence from our studies of the response of the SCC to CPZ, as well as evidence gathered by other workers in other tissues, leads us to suspect that the rise in SCC follows an increase in membrane permeability. CPZ produces an increase in transepithelial electrical conductivity. The increase in electrical conductivity is a primary response of the tissue to CPZ; it is not secondary to the rise in transepithelial sodium transport, for it is seen in the absence of such transport. When sodium in the mucosal solution is replaced with choline, the conductance of the tissue falls and the SCC disappears. Under these conditions, the increase in electrical conductivity normally seen with CPZ is still evident; its magnitude is comparable to that seen in the presence of sodium. According to the current model of the toad bladder, the permeability barrier affected by CPZ should be located at or near the mucosal surface; increased entry of sodium across this surface would result in stimulation of active sodium transport.

Kwant and van Steveninck[14] have shown that exposure of red blood cells to CPZ leads to an increase in intracellular sodium content. They postulate that CPZ alters the "rigidity" of the red blood cell membrane and permits sodium to leak in, a concept consistent with that proposed for the toad bladder.

An alternate possibility which cannot yet be excluded is that CPZ stimulates the sodium pump directly or increases its energy supply. Thus, CPZ might stimulate bladder $Na^+K^+Mg^{2+}$ adenosine triphosphatase (ATPase) or raise intracellular ATP levels. However, no evidence for these possibilites has been obtained in studies of other tissues. CPZ had no effect on the ATP concentration in human erythrocytes,[14] and the drug actually inhibits $Na^+K^+Mg^{2+}$ ATPase in brain preparations.[15]

Vasopressin is also thought to stimulate sodium transport by increasing the permeability of a mucosal barrier.[16] Since the combination of CPZ and vasopressin (the latter present at maximal or near maximal effective concentration) resulted in a greater stimulation than vasopressin alone, the two substances may increase mucosal permeability differently. This is further suggested by the observation that the effect of vasopressin on ionic permeability has so far been shown to be specific for sodium ion, and undirectional (mucosal to serosal) only;[17] furthermore, with sodium replaced by choline musosally, vasopressin has a minimal effect on d.c. resistance.* In contrast, in the latter situation, CPZ substantially reduced d.c. resistance and, with the induction of osmotic water flow, grossly increased permeability to both sodium and potassium occurred. Another important difference between the two compounds is that only vasopressin greatly augments osmotic water flow, even though this action may be attributable to the same effect which produces increased mucosal permeability to sodium.[18]

The studies of water flow provide further evidence that CPZ alters membrane permeability. Transepithelial bulk water flow in the presence of CPZ results in an irreversible solute leak. The solute leak is not seen in the absence of water flow; it is not produced by CPZ, vasopressin or cyclic 3′,5′-AMP alone; nor is it seen when the bladder is challenged with CPZ and vasopressin in the absence of an osmotic gradient. The accumulation of solute reflects an increase in transmembrane permeability to sodium and potassium and presumably chloride; this must have been so, because the only other potential source of solute, the tissue, contains much less Na^+ in its entirety

* R. H. Maffly, unpublished observations.

than appeared in the mucosal bath.[19] We conclude that bulk water flow in the presence of CPZ appears to impair irreversibly the integrity of the bladder cell membranes.

Finally, we would consider the mechanism by which CPZ interferes with osmotic water flow across the toad bladder. When an osmotic gradient is induced across this tissue, in the absence of vasopressin very little net movement of water occurs, i.e. the osmotic permeability is low. Vasopressin added to the serosal bathing medium induces the bulk movement of water along the osmotic gradient.[20] We have considered various explanations for the inhibition of this effect by CPZ.

(1) CPZ might interfere with the action of the hormone by inactivating it or by blocking its effects on permeability in some other way. However, CPZ interferes with the effect of vasopressin when added to the mucosal bath, even though vasopressin is in the other (serosal) bath; hence inactivation, at least outside the tissue, is excluded. Interference with a step between vasopressin and cyclic 3′,5′-AMP is excluded, since CPZ interacts with the latter compound in a manner comparable to its interaction with vasopressin. More importantly, the effect on solute permeability and the enhancement of the SCC when both drug and hormone are present indicate that vasopressin is exerting an action on the tissue even though water flow is reduced.

(2) CPZ might reduce the diffusional permeability of the bladder to water and thereby reduce osmotic permeability.[18] But since the permeability of the tissue to (bigger) solute molecules is markedly increased at the same time, a reduced diffusional permeability to water would seem most unlikely. Our experiments cannot exclude the possibility, however, that the diffusion permeability to water of a critical part of the tissue, or in series with a porous barrier,[18] might be reduced even though permeability elsewhere is simultaneously increased.

(3) The rapid passage of solute molecules into the dilute mucosal bathing medium might dissipate the osmotic gradient. That this was not the case was demonstrated by the following: Water loss was recalculated as flow per measured osmotic gradient for the 30–60 min interval; this corrected flow rate was 47 $\pm$ 11 per cent less in the CPZ-treated hemibladders. Furthermore, when the mucosal bathing medium was replaced with fresh distilled water and the bags were placed in fresh sodium Ringer's solution to restore the original osmotic gradient, inhibition of water flow persisted.

(4) The loss of semipermeability of the membrane, that is, the decrease in the reflection coefficient for solute,[21,22] might result in a reduced driving force for osmotic water flow. It is clear that a gross increase in solute permeability occurred in this situation. The increase was 13–14 times greater with vasopressin plus CPZ than with vasopressin alone. So it seems undeniable that at least some decrease in water flow can be attributed to a decrease in the reflection coefficient. However, there is compelling evidence that this is not the full explanation. The correlation between water flow and solute leak was only fair. The magnitude of the two quantities showed a quite variable relationship from tissue to tissue. Also, in other osmotic experiments with CPZ comparing two concentrations of vasopressin, vasopressin at 100 mU/ml produced twice the solute leak as vasopressin at 10 mU/ml, yet water flow at both concentrations was the same. Finally, at pH 6·3, solute accumulation began 60 min before a decrease in water flow; in the 30–60 min interval, the rate of solute accumulation was near maximal, yet water flow was not diminished.

(5) By exclusion, therefore, we conclude that the reduction in water flow produced by CPZ is attributable in substantial measure to a reduction in the coefficient of osmotic

flow, e.g. a reduction in the net effective size of "pores" through which water flows. More precise quantification would be possible by the performance of studies specifically designed for this end.[22]

It is possible to reconcile these various effects with a number of hypotheses to explain the mechanism of action of CPZ in the toad bladder. We offer one in the framework of current models for sodium transport[6] and water flow[18] across this tissue. We would place the site of action of CPZ in the area of the tight junction between the epithelial cells.[23,24] By reducing the integrity of this barrier to the intercellular movement of ions, CPZ would reduce the d.c. resistance of the membrane. If this action at the same time increased the ionic permeability of the adjacent cell membrane, increased cellular entry of sodium ions from the mucosal bathing medium might increase the rate of active sodium transport. This action would be independent of the permeability effects of vasopressin if the latter acted only along the mucosal surface proper. In the presence of an osmotic gradient and vasopressin, water traverses the mucosal surface and enters the cell. Some passes into the intercellular spaces where it accumulates.[25] Ordinarily, with an intact tight junction, the water leaves the space by passing toward the serosal medium. With the reduced integrity of the tight junction in the presence of CPZ, the distention with water might further disrupt the tight junction and lead to flow of water from the intercellular space back into the mucosal medium. These concepts are consonant with those recently proposed for isolated renal collecting tubules.[26] Hence, net osmotic flow would be reduced and solute permeability would be grossly increased. Morphologic studies will permit evaluation of this hypothesis.

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