Biochemical Pharmacology, Vol. 27, pp. 2781–2786.

MECHANISM OF CHLORPROMAZINE ACTION ON RAT INTESTINAL TRANSPORT PROCESSES

PAVUR R. SUNDARESAN and LEONOR RIVERA-CALIMLIM
Department of Pharmacology and Toxicology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, U.S.A.

(*Received* 20 *April* 1977; *accepted* 5 *October* 1977)

Abstract—In a previous study, it was observed that chlorpromazine (CPZ) inhibited the transport of L-methionine in the rat intestine. The present experiments were designed to delineate the possible mechanism of this effect. Pre-incubation studies with CPZ demonstrated that the tissue concentration of CPZ was much more important than the medium concentration in inhibiting L-methionine transport. This seemed to favor an 'indirect' or 'within-cell' effect (metabolic inhibition or inhibition of sodium and potassium ion-stimulated adenosine triphosphatase [$(Na^+ + K^+)$-ATPase]) over a 'direct' or 'surface' effect (an effect on passive membrane permeability or at the carrier level). CPZ did not affect diffusional entry of L-methionine at the intestinal brush border. The inhibition of non-diffusional mucosal transport of L-methionine by CPZ was not competitive; CPZ inhibited D-galactose transport in the same time-dependent manner as it inhibited L-methionine transport. No CPZ effect on carrier-mediated diffusion of D-xylose was observed. Thus, CPZ effect on active transport processes in the intestine seems to be mediated predominantly within the cell; from the present study it is not clear whether this effect is through inhibition of metabolic energy or inhibition of $(Na^+ + K^+)$-ATPase.

The effect of chlorpromazine (CPZ), the phenothiazine tranquilizer, on intestinal transport processes has been the focus of recent studies from our laboratory. Orally administered CPZ in rats was found to inhibit intestinal transport of L-methionine but not that of D-xylose [1]. The inhibitory effect of CPZ on L-methionine transport was shown to be time- and concentration-dependent, and was associated with both unchanged CPZ and some CPZ metabolites [2].

The present investigation defines the mechanism of the CPZ effect on intestinal transport. The experiments were based on the following rationale. There are four ways by which CPZ could inhibit L-methionine transport in the intestine: (a) it could bind and inactivate the L-methionine carrier, (b) it could diminish membrane permeability of the brush border to L-methionine, (c) it could inhibit glycolysis and/or oxidative phosphorylation in the mucosal epithelial cell and thus decrease the energy supply; since the active transport processes are coupled to the energy source either directly or indirectly [3, 4], this would result in decreased transport, and (d) it could inhibit the activity of sodium and potassium ion-stimulated adenosine triphosphatase [$(Na^+ + K^+)$-ATPase], which provides energy for active non-electrolyte transport in the intestine [3, 4]. The first two of these mechanisms necessarily involve the surface of the mucosal epithelial membrane. Thus, these 'direct' or 'surface membrane' effects would be expected to come on at an early time and be influenced much more by the medium concentration of CPZ than by the tissue concentration of CPZ. In contrast, the last two mechanisms ('indirect' or 'within-cell' effects*) would be expected to have a slow onset and to be influenced more by the tissue concentration of CPZ than medium concentrations of CPZ. Thus, the time course of the inhibitory effect would give an indication of the mechanism of the CPZ effect in the intestine. Also, since the nature and specificity of the above four mechanisms would be expected to differ, by defining the nature and specificity of the CPZ effect and comparing it with the effects of inhibitors with known mechanisms of action one should get further insight into the mechanism of the CPZ effect.

MATERIALS AND METHODS

Materials. CPZ HCl was generously donated by Smith, Kline & French Laboratories, Philadelphia, PA. ^{14}C-ring-labeled CPZ HCl was obtained from Applied Science Laboratories, Inc., State College, PA. The following materials were obtained from Sigma Chemical Co., St. Louis, MO: L-methionine, D-xylose, D-galactose, L-leucine (substantially free of isoleucine and methionine), Trizma base, ouabain and 2,4-dinitrophenol (DNP). L[1-^{14}C]-methionine, D[1-^{14}C]galactose, D[U-^{14}C]xylose, and NCS (Nuclear-Chicago Solubilizer) tissue solubilizer were obtained from Amersham/Searle Corp., Arlington Heights, IL. All other chemicals were purchased from Fisher Scientific Co., Rochester, NY, and were at least of reagent grade purity. The purity of labeled and unlabeled compounds used in the study was at least 98 per cent.

Animals. Male Sprague–Dawley rats (250–375 g), maintained on regular Purina Chow laboratory diet, were used in all the experiments. They were deprived of food for 14–18 hr before sacrifice but had free access to water.

* Most of the evidence in the literature indicates that most, if not all, $(Na^+ + K^+)$-ATPase in the intestinal cell is present in the basolateral membrane [5–8]. Thus, for action through an effect on $(Na^+ + K^+)$-ATPase, entry into the tissue would be needed.

General experimental procedure. The procedures have been described in detail elsewhere [2]. Rats were killed by stunning and decapitation. Intestinal segments, approximately 6 cm long, were taken from the mid-jejunum and everted by the method of Wilson and Wiseman [9]. Each segment was filled with 0.5 ml buffer (containing 5 mM glucose as metabolic substrate), ligated, and placed in 25 ml of the incubation buffer in 50-ml Erlenmeyer flasks. In most of the experiments, modified Krebs–Tris buffer (pH 7.0 at 37°) gassed with 100% oxygen was used. Transport substrate and other drugs were added to the mucosal side only. Incubations were done under an atmosphere of oxygen at 37° in a Dubnoff metabolic incubator shaking at 100 oscillations/min. At the end of the incubation period, the sacs were quickly removed and rinsed in ice-cold buffer having the same composition as the incubation buffer but lacking transport substrate and drug. The sacs were cut open and the serosal fluid was collected. Each sac was placed in a pre-weighed scintillation vial and dried at 105° overnight. The vial with the sac was then reweighed to get the dry tissue weight. The tissues were solubilized in 1.5 ml of tissue solubilizer.

No osmolality adjustments were made unless the difference between the inside and outside buffers exceeded 10 mOsmoles. When 40 mM leucine was used in the incubation buffer (leucine–Krebs–Tris) the osmolality was adjusted by reducing the NaCl in the buffer (in these cases the fluid inside the sac was the Krebs–Tris buffer containing 5 mM glucose). When required, the pH was adjusted after drug addition with 1 N NaOH or 1 N HCl.

Procedure for pre-incubation studies. Pre-incubations were done in 25 ml of the appropriate buffer contained in 50-ml Erlenmeyer flasks. All pre-incubations were carried out at 37° in the Dubnoff incubator. At the end of pre-incubation period, the sacs were taken out, quickly rinsed and then transferred to the appropriate flask for incubation as described under "General experimental procedure".

Special precautions were taken to minimize photo-oxidation of samples containing CPZ, as described [2].

Determination of radioactivity. Radioactivity was counted in 10 ml Triton X-100–toluene scintillation liquid in a Packard Tricarb scintillation spectrometer [10].

Expression of data. The parameters used were the following: (a) Mucosal transport:

$$\frac{\text{Amount of substance in tissue + amount of substance in serosal compartment}}{\text{g of dry tissue}}$$

(b) Serosal transfer:

$$\frac{\text{Amount of substance in serosal compartment}}{\text{g of dry tissue}}$$

(c) Tissue content:

$$\frac{\text{Amount of substance in tissue}}{\text{g of dry tissue}}$$

(d) Per cent control value:

$$\frac{\text{Mean mucosal transport for treated at time } t}{\text{Mean mucosal transport for control at time } t} \times 100$$

(e) $\Delta t_2 - t_1$ Per cent control value:

$$\frac{\begin{array}{c}(\text{Mean mucosal transport for treated at time } t_2) \\ - \\ (\text{mean mucosal transport for treated at time } t_1)\end{array}}{\begin{array}{c}(\text{Mean mucosal transport for control at time } t_2) \\ - \\ (\text{mean mucosal transport for control at time } t_1)\end{array}} \times 100.$$

RESULTS

CPZ pre-incubation and effect of L-methionine transport. These studies were designed to bring out the relative importance of the medium and tissue CPZ concentrations* in producing the inhibitory effect on L-methionine transport. Two approaches were used:

First, sacs were pre-incubated in Krebs–Tris buffer containing 1 mM CPZ (treated) or in CPZ-free Krebs–Tris buffer (control). During the incubation period when L-methionine mucosal transport was monitored, the medium contained 1 mM CPZ in both cases. If the medium CPZ concentration was the important factor in producing the inhibitory effect, no difference in L-methionine mucosal transport should be noted between the treated and the control groups. If the tissue CPZ concentration was important, the treated group should show less methionine transport than the controls. As can be seen (Table 1), CPZ pre-incubated sacs showed significantly less mucosal transport of L-methionine than controls. It is possible that CPZ remaining on

Table 1. Comparison of L-methionine mucosal transport in CPZ pre-incubated and control sacs with incubations done in the presence of CPZ*

Group	Mucosal transport (μmoles/g dry wt)	
	5 min	15 min
Treated	3.7 ± 0.3†	6.2 ± 0.3‡
Control	4.7 ± 0.3	9.2 ± 0.7
Per cent control	78.9	67.4

Δ_{5-0} Per cent control = 78.9
Δ_{15-5} Per cent control = 55.6

* Sacs were pre-incubated for 15 min in 25 ml of standard Krebs–Tris buffer with (treated) or without (control) 1 mM CPZ. After pre-incubation, sacs were transferred (time 0) to 25 ml Krebs–Tris incubation medium containing 1 mM L-methionine (including 0.4 μCi L[^{14}C]methionine) and 1 mM CPZ. Incubations were terminated at the indicated times and values of the mucosal transport determined. Other experimental details are described in Materials and Methods. Values are the means ± S.E. from four sacs.

† $P < 0.05$.

‡ $P < 0.01$.

* CPZ tissue uptake with time has been described in our earlier studies [2].

Table 2. Comparison of L-methionine mucosal transport in CPZ pre-incubated and control sacs with incubations done in CPZ-free medium*

Group	Mucosal transport (μmoles/g dry wt) 5 min	15 min
Treated	4.5 ± 0.2†	9.1 ± 0.6‡
Control	6.5 ± 0.6	12.9 ± 1.1
Per cent control	69.2	70.5

Δ_{5-0} Per cent control = 69.2
Δ_{15-5} Per cent control = 71.9

* Experimental conditions were as described in the legend to Table 1 except that the incubation medium did not contain CPZ. Values are the means ± S.E. for six sacs.
† $P < 0.01$.
‡ $P < 0.05$.

the surface of the CPZ pre-incubated sacs may have produced some 'additional inhibitory' effect. However, since the sacs were rinsed in the transfer process, this amount would be expected to be minimal. Also any possible effect this factor might have should be nullified within the first 5 min of incubation. Thus, the observed results of (a) decreased per cent control values at 15 min as compared to 5 min (indicating increasing inhibition with time), and (b) decreased Δ_{15-5} per cent control value as compared to Δ_{5-0} per cent control value (indicating that less mucosal transport occurred in the treated as compared to control in the 5–15 min period than in the 0–5 min period) strongly indicate the importance of tissue CPZ concentration over medium CPZ concentration.

Second, sacs were pre-incubated in Krebs–Tris buffer containing 1 mM CPZ (treated) or in CPZ-free Krebs–Tris buffer (control). During the incubation period, no CPZ was present in either medium. If medium CPZ concentration was important, no difference in transport should be noted between the two groups. If tissue CPZ concentration was important, the treated group should show less transport than the control. It is clear (Table 2) that CPZ pre-incubation did significantly decrease the L-methionine transporting capabilities of the sacs.

CPZ effect on L-methionine diffusion. As discussed earlier, one way CPZ could affect L-methionine transport in the intestine was by affecting the passive permeability of the brush border membrane to L-methionine. To investigate this possibility, diffusion was measured based on the following rationale.

L-Methionine mucosal transport occurring at the brush border can be considered to be made up of a carrier-mediated active component and a diffusion component. If the carrier-mediated transport of L-methionine can be specifically blocked, then whatever transport is seen to occur under those conditions would represent the diffusion component. While no inhibitors are available which are specific for amino acid carrier, this problem could be circumvented in cases of relatively low medium concentrations of L-methionine by using another amino acid known to use the same neutral amino acid pathway as L-methionine in concentrations high

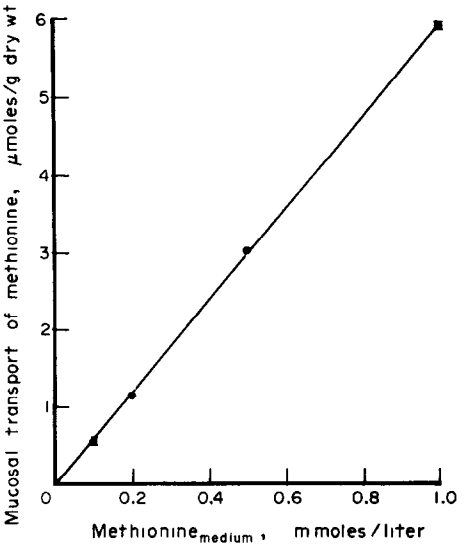

Fig. 1. L-Methionine mucosal transport by everted sacs from different medium concentrations of L-methionine in the presence of 40 mM L-leucine. Everted sacs were incubated in 25 ml of leucine–Krebs–Tris buffer containing 40 mM L-leucine in the presence of 0.1, 0.2, 0.5 and 1 mM L-methionine (including 0.4 μCi L[^{14}C]-methionine). Incubations were terminated at 30 min and mucosal transport values were determined as described in the text. Each point represents the mean of results obtained from two sacs. Individual values did not vary more than 10 per cent from the means shown.

enough to block off almost all carrier-mediated entry of L-methionine. This principle has been used in mucosal strips and isolated cells to study diffusion of L-methionine and other neutral amino acids [11, 12].

In the present study, L-leucine (which uses the same neutral amino acid pathway of L-methionine in the rat intestine [13]) was used in a concentration of 40 mM to block off all carrier-mediated entry of L-methionine. Figure 1 shows some evidence that, in the presence of 40 mM L-leucine, the observed L-methionine mucosal transport represented primarily diffusion; the L-methionine mucosal transport was a linear function of the substrate concentration between 0.1 and 1 mM and linearity is consistent with primarily diffusional entry.

Table 3 shows the time-course of the effect of

Table 3. Effect of CPZ on diffusional L-methionine mucosal transport*

Group	Diffusional L-methionine mucosal transport (μmoles/g dry wt) 5 min	15 min	30 min
Treated	2.2 ± 0.2	3.8 ± 0.4	5.7 ± 0.5
Control	2.2 ± 0.3	3.8 ± 0.3	6.2 ± 0.6

* Everted sacs were incubated in 25 ml of leucine–Krebs–Tris buffer containing 1 mM L-methionine (with 0.4 μCi L-[^{14}C]methionine) with (treated) or without (control) 1 mM CPZ. Incubations were terminated at indicated times and mucosal transport values determined as described in Materials and Methods. Each value represents the mean ± S.E. of four sacs.

Table 4. Effect of CPZ on L-methionine mucosal transport occurring from different medium concentrations of L-methionine*

Methionine in medium (M)	Group	Total L-methionine mucosal transport (μmoles/g dry wt)			Non-diffusional L-methionine muscosal transport (μmoles/g dry wt)
		5 min	15 min	30 min	30 min
1×10^{-4}	Treated	0.43 ± 0.02	0.92 ± 0.13	1.40 ± 0.12	0.8
	Control	0.66 ± 0.05	1.52 ± 0.18	2.72 ± 0.36	2.1
	Per cent control	65.2	60.5	51.5	38.1
1×10^{-3}	Treated	3.6 ± 0.3	6.6 ± 0.3	9.8 ± 0.9	3.9
	Control	4.4 ± 0.3	10.6 ± 1.4	18.8 ± 1.5	12.9
	Per cent control	81.8	62.3	52.1	30.2
1×10^{-2}	Treated	24.5 ± 0.8	43.8 ± 3.7	62.9 ± 3.3	3.9
	Control	26.3 ± 2.0	47.6 ± 2.8	84.8 ± 3.7	25.8
	Per cent control	93.2	92.0	74.2	15.1

* Everted sacs were incubated in 25 ml of Krebs–Tris buffer containing various concentrations of L-methionine (including 0.4 μCi L[^{14}C]methionine) with (treated) or without (control) 1 mM CPZ. Other details are as described for Table 3. Each value is the mean ± S.E. of at least five sacs. Values of 30-min non-diffusional L-methionine mucosal transport (shown at last column) were obtained by subtracting the values of diffusional L-methionine mucosal transport at different medium methionine concentrations (obtained by extrapolation from Fig. 1) from the corresponding total mucosal transport values shown in this table.

CPZ on the mucosal transport of L-methionine in the presence of 40 mM L-leucine. CPZ had no significant effect at any of the time points.

Nature of CPZ inhibition of L-methionine transport. A single medium CPZ concentration (1 mM) was used with various concentrations of L-methionine (up to 100-fold; see Table 4). The decreasing percentage of inhibition with increased methionine medium concentration seems to indicate competitive inhibition. However, these data are uncorrected for diffusional entry of L-methionine occurring at different medium concentrations. Since, as shown above (Table 3), CPZ does not affect diffusional entry of L-methionine, this factor must be subtracted from the treated and control values at each time point for each L-methionine concentration before the effect of CPZ on the non-diffusional mucosal transport of L-methionine can be assessed.

At all L-methionine concentrations used, the inhibitory effect of CPZ was most clear at 30 min; calculations for nondiffusional L-methionine mucosal transport values were made for this time point, as described in Table 4. Increasing the concentration of L-methionine did not antagonize the inhibitory effect of CPZ on nondiffusional L-methionine mucosal transport.

Specificity of the CPZ effect. To define the specificity of the CPZ inhibitory action, its effect on the monosaccharide transport system was studied. Monosaccharide transport in the intestine is thought to occur by a carrier system completely different from the amino acid carrier system [3]. D-Galactose, which is actively transported in the rat intestine, and D-xylose, which is transported predominantly by carrier-mediated diffusion [14–16], were used in the study.

CPZ caused a time-dependent inhibition of D-galactose (Fig. 2). The pattern and time–course were similar to those found with L-methionine (see Table 4).

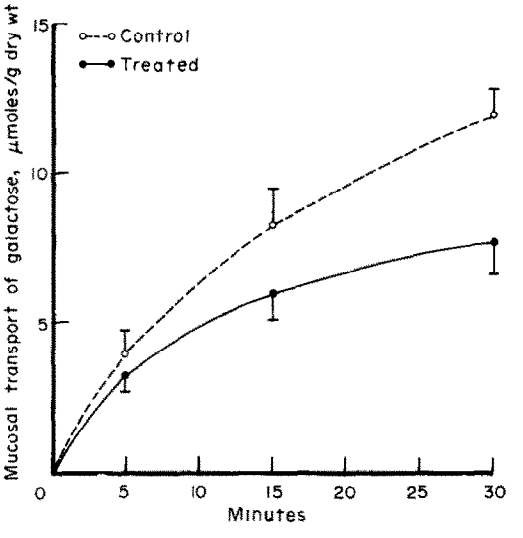

Fig. 2. Time–course of CPZ effect on D-galactose mucosal transport by everted sacs. Everted sacs were incubated in 25 ml of standard Krebs–Tris buffer containing 1 mM D-galactose (including 0.4 μCi D[^{14}C]galactose) with (treated) or without (control) 1 mM CPZ. For other details see Table 3. Each point represents the mean ± S.E. of results obtained from four sacs.

CPZ did not significantly affect the mucosal transport of D-xylose. With a 10 mM D-xylose concentration in the medium, the 30-min mucosal transport values were, respectively, 49.5 ± 1.3 and 54.4 ± 0.9 μmoles/g of tissue for the treated and control groups. The CPZ tissue uptake studied showed that this lack of inhibitory effect on D-xylose transport was not due to D-xylose inhibition of CPZ uptake by the intestinal tissue.*

* Unpublished observations.

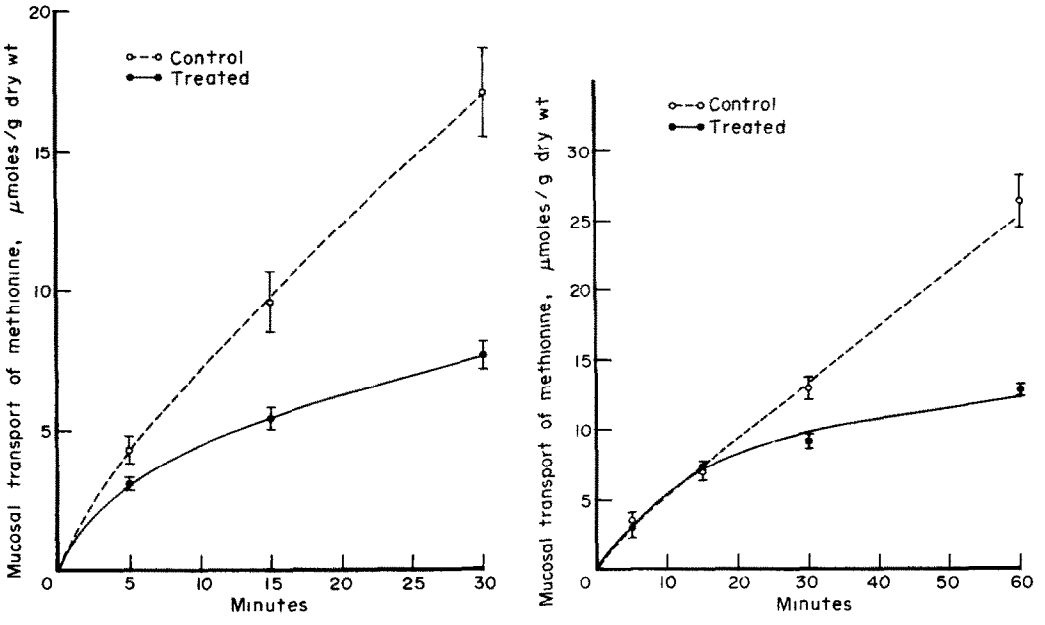

Fig. 3. Time-course of the effects of DNP and ouabain on L-methionine mucosal transport by everted sacs. Everted sacs were incubated in 25 ml of standard Krebs–Tris buffer containing 1 mM L-methionine (including 0.4 μCi L[^{14}C]methionine) with (treated) or without (control) the appropriate inhibitor. Other details were similar to those described in Table 3. Each point represents the mean ± S.E. of results obtained from at least two sacs. Panel A: DNP, 1 mM in the medium; panel B: ouabain, 10 mM in the serosal fluid.

Comparison of the CPZ effect with DNP and ouabain. CPZ inhibition of L-methionine transport in the intestine was compared with that produced by DNP, which inhibits active transport by uncoupling oxidative phosphorylation [3] and ouabain, a specific inhibitor of ($Na^+ + K^+$)-ATPase [3]. Figure 3 shows the time-course of the effects of the different inhibitors on L-methionine transport. The CPZ time-course (Table 4; see also Fig. 2 in Ref. 2) showing increasing percentage of inhibition with time was most like that of DNP (Fig. 3A). The effect of ouabain came at a relatively late time point (Fig. 3B).

DISCUSSION

CPZ inhibition of L-methionine transport in the intestine could be brought about by any one of the following actions: (a) inhibition at the carrier level, (b) effect on membrane permeability, (c) decreased glycolysis and/or oxidative phosphorylation, and (d) inhibition of ($Na^+ + K^+$)-ATPase. CPZ has been reported to exhibit each of these actions in some tissue. The CPZ effect on glucose passage in the erythrocyte has been reported to be at the glucose carrier level [17, 18]. Many of the effects of CPZ on transport processes in the red blood cell, liver and brain are thought to be exerted through effects on passive membrane permeability [19–24]. CPZ inhibits oxidative phosphorylation and glycolytic enzymes in many tissues [23]. Inhibition of ($Na^+ + K^+$)-ATPase in the brain by CPZ or CPZ free radical has also been reported [25–27].

In the present study on the rat intestine, the results of the CPZ pre-incubation studies strongly suggested that the tissue concentration of CPZ was more important than the medium concentration for the inhibition of L-methionine transport. This seemed to favor indirect or within-cell mechanisms, i.e. an effect on the energy production system or ($Na^+ + K^+$)-ATPase, over surface effects (effects on carrier or membrane permeability). The studies on L-methionine diffusion indicated that CPZ does not affect passive membrane permeability of the brush border membrane to L-methionine. CPZ inhibition of nondiffusional transport of L-methionine was not competitive, as demonstrated by studies with various concentrations of L-methionine in the presence of a fixed medium CPZ concentration. CPZ inhibited D-galactose and L-methionine in the same time-dependent manner, suggesting that the CPZ effect is mediated through a factor common to both the amino acid and the monosaccharide transport systems. Inhibition at the carrier level seemed unlikely since neutral amino acid and sugar carrier systems are generally considered distinct from each other [3].

The lack of a CPZ effect on D-xylose transport further supported the idea that the CPZ effect was not mediated at the carrier level. D-Xylose is transported in the rat intestine predominantly by carrier-mediated diffusion; phloridzin can almost completely block the carrier-mediated diffusion; phloridzin can almost completely block the carrier-mediated diffusion of D-xylose [28]. Thus, if CPZ inhibition of D-galactose transport was at the carrier level, it would produce inhibition of D-xylose transport. Thus, the overall evidence from the present study indicates that the CPZ effect on intestinal transport processes is predominantly exerted by indirect or 'within cell' effects. From the results of the study, it is not possible to assess whether the CPZ effect is on cell energy production (glycolysis

or oxidative phosphorylation) or $(Na^+ + K^+)$-ATPase. The ouabain inhibitory effect was seen to occur at a later time than either the DNP or the CPZ effect; however, this might be more related to the accessibility of ouabain to the site of action than to anything else. Ouabain is known to be much less potent in the rat intestine than in the intestines of many other animals [3, 29].

Acknowledgements—We thank Susan Kelvie and Alan Siracusa for skilled technical assistance. We are grateful to Drs. George Kimmich, J. Crispin Smith and Louis Lasagna for their critical comments and suggestions throughout the course of the work and for their review of the final manuscript. Support for this work came in part from Grants GM-15190 and MH-24188 from the U.S. Public Health Service. Fellowship support to Dr. Sundaresan was kindly provided by Abbott Universal Ltd.

REFERENCES

1. P. R. Sundaresan and L. Rivera-Calimlim, *J. Pharmac. exp. Ther.* **194,** 593(1975).
2. P. R. Sundaresan and L. Rivera-Calimlim, *Biochem. Pharmac.* **26,** 1411 (1977).
3. G. A. Kimmich, *Biochim. biophys. Acta* **300,** 31 (1973).
4. S. G. Schultz and P. F. Curran, *Physiol. Rev.* **5,** 637 (1970).
5. J. P. Quigley and G. S. Gotterer, *Biochim. biophys. Acta* **173,** 456 (1969).
6. M. Fijita, H. Matsui, K. Nagano and M. Nakao, *Biochim. biophys. Acta* **344,** 404 (1971).
7. M. Fujita, M. Ohta, K. Kawai, H. Matsui and M. Nakao, *Biochim. biophys. Acta* **274,** 336 (1972).
8. S. G. Schultz, R. A. Frizzell and H. N. Nellans, *A. Rev. Physiol.* **36,** 51 (1974).
9. T. H. Wilson and G. Wiseman, *J. Physiol., Lond.* **123,** 116 (1954).
10. J. C. Turner, *Int. J. appl. Radiat. Isotopes* **19,** 447 (1968).
11. P. M. Burrill, P. A. Sattelmeyer and J. Lerner, *Biochim. biophys. Acta* **373,** 265 (1974).
12. A. M. Tucker and G. A. Kimmich, *J. memb. Biol.* **2,** 1 (1973).
13. H. Newey and D. H. Smyth, *Nature, Lond.* **202,** 300 (1964).
14. R. K. Crane, *Physiol. Rev.* **40,** 789 (1960).
15. D. H. Smyth, in *Intestinal Transport of Electrolytes, Amino Acids and Sugars* (Eds. W. M. Armstrong and A. S. Nunn), p. 52. Charles C. Thomas, Springfield, IL (1971).
16. T. H. Wilson, in *Sugars* (Ed. T. H. Wilson), p. 69. W. B. Saunders, Philadelphia (1962).
17. G. F. Baker and H. J. Rogers, *Biochem. Pharmac.* **21,** 1871 (1972).
18. G. F. Baker and H. J. Rogers, *J. Physiol., Lond.* **232,** 547 (1973).
19. E. Dybing, *Acta pharmac. tox.* **31,** 287 (1972).
20. E. Dybing, *Biochem. Pharmac.* **22,** 591 (1973).
21. E. Dybing, *Biochem. Pharmac.* **22,** 1981 (1973).
22. C. Crifo, A. Bozzi, B. Mondovi and R. Strom. *Biochem. Pharmac.* **22,** 2511 (1973).
23. P. S. Guth and M. A. Spirtes, *Int. Rev. Neurobiol.* **7,** 231 (1964).
24. P. Seeman, W. O. Kwant, M. Goldberg and M. Chau-Wong, *Biochim. biophys. Acta* **241,** 349 (1971).
25. T. Akera and T. M. Brody, *Molec. Pharmac.* **4,** 600 (1968).
26. T. M. Brody, T. Akera, S. I. Baskin, R. Gubitz and C. Y. Lee, *Ann. N.Y. Acad. Sci.* **242,** 527 (1974).
27. J. D. Robinson, J. Lowinger and B. Bettinger, *Biochem. Pharmac.* **17,** 1113 (1968).
28. F. Alvarado, *Biochim. biophys. Acta* **112,** 292 (1966).
29. H. Newey, P. A. Sanford and D. H. Smyth, *J. Physiol., Lond.* **194,** 237 (1968).