Biochemical Pharmacology, Vol. 26, pp. 763–767, Pergamon Press, 1977. Printed in Great Britain.

INTESTINAL TRANSFER OF THE QUATERNARY AMMONIUM COMPOUND *N*-METHYL-SCOPOLAMINE BY TWO TRANSPORT MECHANISMS IN SERIES*

KLAUS TURNHEIM†, FRITZ LAUTERBACH‡ and NORBERT KOLASSA†

†Pharmakologisches Institut der Universität Wien, A-1090 Vienna, Währingerstraße 13a, Austria and ‡Institut für Pharmakologie und Toxikologie der Ruhr-Universität Bochum, D-463 Bochum-Querenburg, Im Lottental, West Germany.

Abstract—In order to localize the previously demonstrated intestinal transport mechanism for quaternary ammonium compounds, counter-transport and efflux experiments were performed with *N*-methyl-scopolamine (NMScop) on isolated jejunal epithelia of guinea pigs. (1) Both [^{3}H]NMScop uptake from and [^{3}H]NMScop release to the blood side of jejunal epithelia were stimulated by the presence of high concentrations of unlabelled NMScop in the compartment, into which flux was occurring. The phenomenon of counter-transport was not apparent at the luminal cell membrane. (2) From preloaded epithelia [^{3}H]NMScop efflux to the lumen side was markedly reduced during NaCN-intoxication as compared to controls, whereas that to the blood side increased. These effects were brought about mainly by a decrease in the luminal transfer coefficient. (3) At the luminal membrane the efflux coefficient far exceeded that for influx, in spite of the negative potential of the cell interior. It is concluded that NMScop is transferred across the jejunal epithelium by two transport mechanisms in series, one located in the basolateral and one in the luminal cell membrane.

In previous *in vitro* and *in vivo* investigations an intestinal secretory system for monoquaternary ammonium compounds, capable of uphill transport, was demonstrated in guinea pig jejunum [1, 2]. Since transcellular permeation involves movements across at least two membranes arranged in series, the question arose in which membrane the transport mechanism for quaternary ammonium compounds is located.

Earlier data on cellular uptake and transcellular permeation of quaternary ammonium compounds during aerobic and anaerobic conditions suggested a cell model consisting of both a luminal and a basolateral carrier, both transporting preferentially towards the gut lumen [1]. It is the aim of the present study to obtain more evidence for the postulated two transport processes.

One of the most compelling arguments for the existence of a carrier is the demonstration of counter-transport, defined as the acceleration of the permeation of a substrate across a membrane by the presence of the same or a chemically related substance on the opposite side of the membrane [3]. This phenomenon was studied both at the luminal and the basolateral membrane of the intestinal epithelium by use of the method of the isolated mucosa of guinea pig jejunum [4, 5].

In a second series of experiments the effect of inhibition of oxydative metabolism on bidirectional substrate efflux from preloaded epithelia was tested and the respective transfer coefficients were calculated.

NMScop was shown to exhibit the highest secretion rate of the compounds previously tested [1] and was therefore chosen for the present study.

* Part of this paper was presented at the FEBS-Symposium on the Biochemistry of Membrane Transport, Zurich, Switzerland, July 18–23, 1976. Abstract P 201.

MATERIALS AND METHODS

Materials

Guinea pigs of both sexes (inbred strain, Fa.Rost, D-581 Witten), ranging in weight from 367 to 548 g and fed a diet (Altromin 3022/MS^R, Altromin GMBH, D-491 Lage/Lippe) enriched with vitamins (Multivitaminsaft Lappe, Lappe-Arzneimittel, D-506 Bensberg) were used for the experiments.

N-[^{3}H]methylscopolamine bromide (107 mCi/mmole) was supplied by C. H. Boehringer & Sohn, D-6507 Ingelheim. [Carboxyl-^{14}C]inulin (1–3 mCi/g) was obtained from New England Nuclear Corp., Boston, MA., USA. All chemicals used were of analytical grade.

Experimental procedure

The method of the isolated mucosa has been described in detail elsewhere [1, 4, 5]. In principle, isolated epithelia from guinea pig jejunum constitute a separating membrane of 0.2 cm^2 area between two plexiglass chambers, which are filled with 200 μl buffered physiological salt solution (composition in mM: 98.3 NaCl, 7.0 KCl, 3.0 $CaCl_2$, 1.0 $MgSO_4$, 0.9 sodium phosphate buffer pH 7.4, 29.4 tris-(hydroxymethyl)-aminomethan buffer pH 7.4, 14.0 glucose, 14.0 mannitol) and supplied with O_2. All experiments were performed at 37°. Preloading of epithelia was performed by administering the substrate from the blood side during a 45 min preincubation period.

Counter-transport experiments. For the study on the phenomenon of counter-transport isolated epithelia were subjected to two successive incubation periods. In the first set of experiments the epithelia were preincubated for 45 min with 1 μM [^{3}H]NMScop, then the preparations were mounted in new chambers and the release of [^{3}H]NMScop within 10 min into incubation medium containing no (controls) or 1 mM unlabelled NMScop on the lumen

or the blood side of the mucosa was measured. In the second set of experiments the uptake within 10 min of 1 μM [^{3}H]NMScop from the blood or the lumen side into epithelia preincubated for 45 min with 1 mM unlabelled NMScop or no NMScop (controls) was tested. The substrate concentrations used for the counter-transport study, 1 μM [^{3}H]NMScop and 1 mM unlabelled NMScop, are either below or above the apparent half-saturation constant of 450 μM found for transepithelial secretion of NMScop [1].

As a marker for extracellular space and paracellular permeation [^{14}C]inulin was added to the incubation medium together with [^{3}H]NMScop.

At the end of the second incubation period the radioactivity in 100 μl incubation medium of both the lumen and the blood side was determined in 9 ml Bray's [6] scintillator by a Packard Scintillation Spectrometer, Tri Carb, Model 3380. In order to determine the tissue radioactivity the mucosa was punched out, weighed (11.82 mg wet wt, S.E.M. = 0.17, n = 123), and soaked in 0.2 ml water for 2 hr at room temperature with subsequent addition of 9 ml Bray's scintillator.

Efflux experiments. For the calculation of the transfer coefficients of both the luminal and the basolateral membrane isolated epithelia were preloaded for 45 min with 10 μM [^{3}H]NMScop. After changing the mucosa to new chambers, the time-course of substrate efflux into 200 μl buffered saline on either side of the epithelium was determined from the radioactivity in 100 μl luminal and basolateral incubation medium, which were withdrawn every 4 min and replaced by the same volume of fresh saline.

Calculations

The cellular amount of substrate, which will be dealt with as the parameter of cellular uptake, was defined as the difference between the total amount in the tissue and the amount in the extracellular space (inulin space), thus including possible cell membrane binding. Transcellular permeation was calculated from the difference between the amounts of substrate and inulin in the counter-compartment, both in per cent of the amount administered.

The compartmental analysis of the efflux experiments and all other calculations were carried out on a pdp 11/10 computer, Digital Equipment Corp., Maynard, MA, U.S.A.

RESULTS AND DISCUSSION

Counter-transport experiments

In the first set of experiments isolated mucosae were preloaded with 1 μM [^{3}H]NMScop. The addition of 1 mM unlabelled NMScop to the blood side stimulated the release of labelled substrate substantially (Table 1): The cellular content after the 10 min efflux period was decreased by approximately 1/3 in comparison to controls. This fall was brought about by a marked increase in basolateral release, whereas that to the lumen decreased. The addition of 1 mM unlabelled NMScop to the lumen side of the mucosa had no significant effect on the rate of [^{3}H]NMScop release.

The observed effects are consistent with the phenomenon of counter-transport at the basolateral membrane of the epithelial cells. But displacement of labelled NMScop from binding sites on the external face of the cells by the excess of unlabelled NMScop may also be responsible for the increase in basolateral outflow.

In order to exclude this possibility experiments with the reverse design were performed: The cellular uptake of 1 μM [^{3}H]NMScop was tested in epithelia which were preloaded with 1 mM unlabelled NMScop, yielding a cellular concentration of approximately 350 μM [1]. Since the cellular NMScop concentration was more than 2 orders of magnitude higher than the [^{3}H]NMScop concentration on the side of administration, displacement of unlabelled NMScop from the external face of the cells cannot account for an increase in cellular uptake of [^{3}H]NMScop.

In preloaded epithelia the cellular uptake of labelled substrate across the basolateral membrane was 50 per cent higher than in not-preloaded controls (Table 2). Yet the cellular uptake from the lumen side was not significantly altered by preloading the epithelia.

Thus in both sets of experiments the phenomenon of counter-transport was clearly evident at the basolateral membrane, strongly indicating the existence of a basolateral carrier mechanism for monoquaternary ammonium compounds. But at the luminal membrane neither uptake nor release of labelled substrate was accelerated by the presence of unlabelled substrate at the trans-side of the cell membrane: hence

Table 1. Counter-transport experiments: Influence of unlabelled NMScop in the incubation medium on the cellular release of [^{3}H]NMScop

	Control	1 mM NMScop on the blood side	1 mM NMScop on the lumen side
Residual cellular [^{3}H]NMScop content (pmoles)	2.15 ± 0.14	1.49 ± 0.11‡	1.99 ± 0.12
[^{3}H]NMScop release (pmoles)			
to the blood side	1.74 ± 0.14	3.08 ± 0.14‡	1.94 ± 0.20
to the lumen side	1.42 ± 0.16	1.06 ± 0.04*	1.40 ± 0.16

Isolated jejunal epithelia, preloaded for 45 min with 1 μM [^{3}H]NMScop. Release of labelled substrate within 10 min into incubation medium containing 1 mM NMScop on the blood or the lumen side in comparison to controls (no NMScop present on either side of the epithelium). Means ± S.E.M. of 18 to 20 experiments.

*,‡ $2P < 0.05$ and 0.001, respectively.

Table 2. Counter-transport experiments: Influence of cellular unlabelled NMScop on the uptake of [^{3}H]NMScop

	Control	Epithelia preloaded with 1 mM NMScop
Cellular uptake of [^{3}H]NMScop (pmoles)		
from the blood side	3.94 ± 0.15	6.02 ± 0.17*
from the lumen side	0.72 ± 0.07	0.80 ± 0.07
Transcellular permeation of [^{3}H]NMScop (pmoles)		
blood-to-lumen	0.76 ± 0.10	0.34 ± 0.04*
lumen-to-blood	0.14 ± 0.04	0.14 ± 0.02

Isolated jejunal epithelia, preloaded for 45 min with 1 mM NMScop or not-preloaded (controls). Cellular uptake and transcellular permeation of label within 10 min after the administration of 1 μM [^{3}H]NMScop to either the blood or the lumen side. Means ± S.E.M. of 14 to 16 experiments.

* $2P < 0.001$.

in the counter-transport study a clear indication for a luminal carrier was not found.

However, considering the transcellular permeation, which under the conditions employed is small in comparison to cellular uptake, it is striking that in mucosae preloaded with 1 mM unlabelled NMScop the permeation of [^{3}H]NMScop in the direction blood-to-lumen was substantially lower than in controls, although the cellular content was markedly enhanced (Table 2). This observation may be explained in terms of inhibition of carrier-mediated transfer of labelled substrate across the luminal membrane by the excess of unlabelled substrate in the cell, whereas in case of mere passive permeability of the luminal membrane an increase in transcellular permeation blood-to-lumen as compared to controls would have to be expected with an increase in cellular concentration.

Efflux experiments

The failure to demonstrate the phenomenon of counter-transport at the luminal membrane does not exclude the existence of a luminal carrier, but may be attributed to a low affinity of the carrier on the outside of the luminal membrane. The assumption of a very low affinity of the luminal transport mechanism for substrate at the outside of the luminal cell membrane as compared to the inside is consistent with the secretory direction of the transport system. But the luminal carrier should become clearly apparent from a study of substrate transfer in the preferential direction of transport, from the cell towards the lumen. A higher permeation rate across the luminal membrane in the direction of efflux as compared to that in the direction of uptake would strongly argue for the presence of a luminal carrier mechanism.

For this investigation the time-course of substrate efflux to both the lumen and the blood side of epithelia preloaded with [^{3}H]NMScop was studied simultaneously either under aerobic conditions or under inhibition of oxydative metabolism with 1 mM NaCN. Within 60 min approximately 90 per cent of the total tissue content was released. The total amount released during infinite incubation time to either the lumen or the blood side was calculated as the sum of all efflux values of the individual sampling periods (see Methods) plus a proportional fraction of the residual radioactivity in the epithelium. As is apparent from a plot of the difference between the amount released to either side of the epithelium during infinite time and that up to time t versus time (Fig. 1), efflux to the lumen as well as to the blood side originates from at least two compartments. Assuming a two compartment model for efflux, the individual rate constants and initial contents of the compartments were calculated by approximating parameter estimates to the experimental data by use of an iterative curve fitting procedure [7].

The rapidly exchanging compartment was defined as extracellular, because both its content and rate

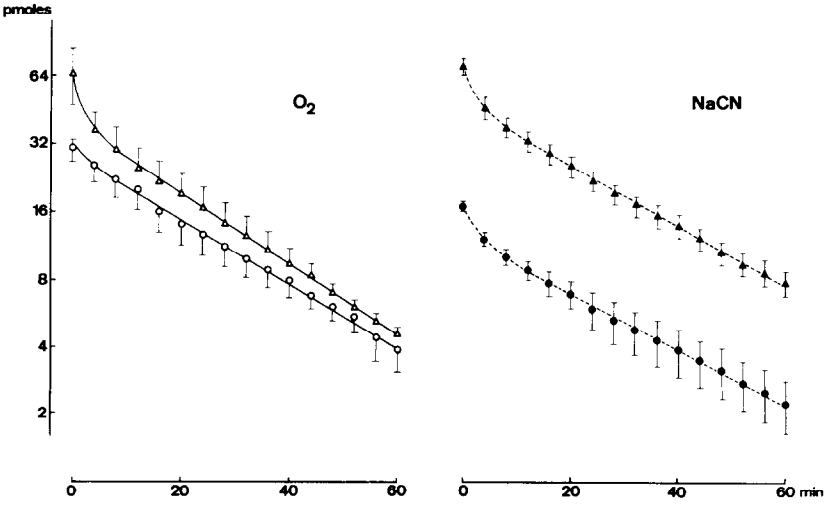

Fig. 1. Efflux experiments. Isolated jejunal epithelia, preloaded for 45 min with 10 μM [^{3}H]NMScop. Time-course of the decrease of the virtual [^{3}H]NMScop tissue pools destined for luminal (circles) or basolateral (triangles) efflux during aerobic conditions (O_2) or intoxication with 1 mM NaCN.

constant approximated the corresponding values obtained from the simultaneously determined [^{14}C]inulin efflux. The slowly exchanging compartment is therefore considered to represent the functional cellular space.

The rate constant of the cellular compartment is equal to $(K_{cl} + K_{cb})$, the sum of the transfer coefficients of the luminal and the basolateral membrane, since the cellular substrate content is decreased by both a luminal and a basolateral elimination process in parallel. A detailed description of an analogous mathematical treatment is given elsewhere [8, 9]. The values for the total amount of cellular substrate released to the lumen and the blood side ($C_{cl(o)}$ and $C_{cb(o)}$, respectively), and $(K_{cl} + K_{cb})$ obtained from the compartmental analysis are given in Table 3.

During aerobic conditions $C_{cb(o)}$ was higher, although not significantly, than $C_{cl(o)}$. This difference was markedly enhanced during NaCN-intoxication, the cellular efflux to the lumen side being greatly reduced, whereas that to the blood side increased (Table 3).

The fact that the values for $(K_{cl} + K_{cb})$ derived from either the luminal or basolateral efflux are almost identical is in agreement with the assumption that both fluxes originate from one cellular compartment.

The transfer coefficients of the individual membranes, K_{cl} and K_{cb}, given in Table 3 were obtained from the relationship $K_{cl}/K_{cb} = C_{cl(o)}/C_{cb(o)}$ [8, 9]. With unimpaired metabolism the luminal transfer coefficient K_{cl} was somewhat smaller than that for the basolateral membrane, K_{cb}. During NaCN-intoxication the luminal transfer coefficient fell to half the value obtained with intact metabolism, whereas the basolateral transfer coefficient rose insignificantly by 27 per cent. The marked increase in total cellular efflux to the blood side observed under NaCN is therefore primarily brought about by the pronounced decrease of the luminal transfer coefficient.

Table 3. Efflux experiments

	O_2	NaCN
$C_{cl(o)} + C_{cb(o)}$ (pmoles)	67.1 ± 14.7	63.4 ± 2.5
$C_{cl(o)}$ (pmoles)	28.9 ± 4.9	13.9 ± 0.7*
$C_{cb(o)}$ (pmoles)	38.2 ± 11.8	49.4 ± 3.0
$(K_{cl} + K_{cb})$ from luminal efflux (10^{-4} min^{-1})	308 ± 11	321 ± 44
$(K_{cl} + K_{cb})$ from basolateral efflux (10^{-4} min^{-1})	323 ± 32	303 ± 18
K_{cl} (10^{-4} min^{-1})	135 ± 20	69 ± 7*
K_{cb} (10^{-4} min^{-1})	187 ± 37	239 ± 16

Isolated jejunal epithelia, preloaded for 45 min with 10 μM [^{3}H]NMScop. From a compartmental analysis of the time-course of substrate efflux to both sides of the mucosa the total amount of cellular [^{3}H]NMScop excreted to the lumen ($C_{cl(o)}$) and the blood side ($C_{cb(o)}$) during infinite incubation, the rate of decrease of the cellular content $(K_{cl} + K_{cb})$, derived from either luminal or basolateral efflux, and the transfer coefficients of the luminal (K_{cl}) and the basolateral membrane (K_{cb}) during aerobic conditions (O_2) and during NaCN-intoxication were estimated from the experimental data given in Fig. 1 by use of an iterative curve fitting procedure. Means ± S.E.M. of 4 experiments.

* $2P < 0.05$.

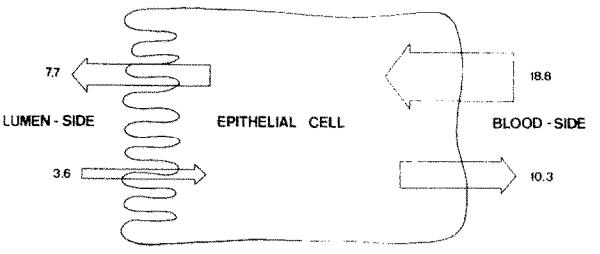

Fig. 2. Model for NMScop transfer across a jejunal epithelial cell. The arrows indicate the apparent flux coefficients (10^{-4} cm min^{-1}) of the individual cell membranes for NMScop concentrations below the apparent K_m for transcellular transport.

The possibility that the observed changes in the transfer coefficients are due to alterations in the passive permeability of the cell membranes does not seem very likely, because the flux across a cell membrane in either direction would have to be influenced in the same manner. But whereas the efflux of [^{3}H]NMScop to the lumen side decreased and that to the blood side increased with NaCN, uptake of [^{3}H]NMScop from the lumen side was enhanced and that from the blood side unchanged, when oxydative metabolism was inhibited by equilibrating the incubation medium with N_2 instead of O_2 [1]. Further, the fact that the two membranes of the epithelial cells were affected by NaCN in a reverse manner argues against changed passive permeability properties of the cell membranes.

Finally, for both membranes the efflux coefficients were compared with the influx coefficients. The efflux coefficients were derived from the transfer coefficients given in Table 3 according to $k = K \times V/A$, and the influx coefficients were calculated from the values of [^{3}H]NMScop uptake in not-preloaded epithelia given in Table 2 according to $k = J/(A \times S)$, where V, A, J, and S are the vol. of the cellular space (with these preparations 11 μl), the cross-sectional area of the epithelium exposed to the incubation medium (0.2 cm^2), the cellular uptake per min and the substrate concentration administered. The resulting cell model with the flux coefficients in the direction of efflux and influx for both cell membranes is depicted in Fig. 2.

As is apparent, the luminal efflux coefficient far exceeds the luminal influx coefficient. Since at the luminal membrane of rabbit [10] and frog [11] intestinal cells a potential difference of 36 mV and 45 mV, respectively, was measured, cell interior negative, the asymmetry of the flux coefficients in spite of an opposing electrical potential substantiates the active nature of the luminal carrier mechanism.

CONCLUDING REMARKS

According to the presented evidence NMScop is transferred across the jejunal epithelium by two carrier mechanism in series, one located in the basolateral and one in the luminal cell membrane.

The existence of the basolateral carrier is clearly apparent from the demonstration of counter-transport: both the uptake from and the efflux to the blood side of the epithelium were stimulated by the presence of substrate in the compartment, into which flux was occurring. The basolateral carrier mechanism was additionally substantiated by the saturability of cellular uptake from the blood side found earlier [1].

The presence of the luminal pump was demonstrated by the finding that the luminal transfer coefficient in the direction of efflux decreased during inhibition of oxydative metabolism (Table 3), whereas luminal uptake increased as compared to aerobic conditions [1]. Furthermore, the predominance of the luminal flux coefficient in the direction of efflux over that for influx (Fig. 2) despite an electrical potential in the reverse direction strongly argues for an extrusive mechanism in the brush-border membrane.

The arrangement of a basolateral and a luminal carrier, both preferentially transporting towards the lumen, explains the earlier observation that the cellular uptake of NMScop from the blood side was unchanged in anaerobiosis in comparison to aerobic conditions, although secretion was markedly inhibited [1]. Assuming a single luminal pump alone inhibition of secretion would have to result in increased cellular uptake, while with a single basolateral carrier alone a decrease in cellular uptake would have to be expected with inhibited secretion. Yet the model with two carrier mechanisms in series complies with the experimental findings: Since less substrate enters and leaves the cells in anaerobiosis, the cellular concentration may remain unaltered as compared to aerobic conditions, although secretion is inhibited.

A three compartment model of the intestinal epithelium without carriers, based on different degrees of dissociation of a weak electrolyte in different compartments due to pH-gradients and different ionic permeabilities of the individual boundaries, was proposed by Jackson and coworkers [12] to explain the higher flux rates of the organic bases benzylamine, hexylamine, and D-amphetamine in the direction serosa-to-lumen than in the opposite direction. This system, however, cannot explain the observations with NMScop, because quaternary ammonium compounds are fixed cations. But considering that the compounds tested by Jackson and coworkers have rather high pK_a-values, ranging between 9 and 10, the findings of these investigators may also be explained by intestinal secretion of these substances in their charged form, using the transport system shown for quaternary ammonium compounds.

Secretion by two transport mechanisms in series does not seem to be unique for quaternary ammonium compounds. A similar system has been inferred for the intestinal secretion of cardiac glycosides [13, 14]. Furthermore, evidence has been presented that the influx of quaternary ammonium compounds into the liver cells as well as their efflux into the bile canaliculi proceed against electrochemical gradients [15, 16]. Thus the arrangement of two transport mechanisms in series might prove to be a widely distributed principle to accelerate transcellular permeation without unduly high intracellular substrate accumulation.

Acknowledgements—The authors express their thanks to C. H. Boehringer & Sohn, D-6507 Ingelheim, and to Fa.Lappe-Arzneimittel, D-506 Bensberg, for the generous supply of labelled and unlabelled *N*-methylscopolamine and of 'Multivitaminsaft Lappe', respectively. The skilful technical assistance of Ms. U. Schnittker and Ms. M. E. Best is appreciated.

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