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# BLOCKING BY 2,4,6-TRIAMINOPYRIMIDINE OF INCREASED TIGHT JUNCTION PERMEABILITY INDUCED BY ACETYLCHOLINE IN THE PANCREAS

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## Summary

1. The permeability of the paracellular pathway in the isolated rabbit pancreas has been studied with the aid of 2,4,6-triaminopyrimidine.

2. Addition of 2,4,6-triaminopyrimidine (1—10 mM) to the bathing medium has no effect on the rate of fluid secretion or on protein, $Na^+$, $K^+$, $Ca^{2+}$ and sucrose concentrations in the secreted fluid.

3. When $1 \cdot 10^{-5}$ M carbachol is also added to the 2,4,6-triaminopyrimidine-containing bathing medium, there is a marked reduction in the increase of the paracellular permeability for sucrose and $Ca^{2+}$ found upon addition of carbachol alone. The enzyme secretion, induced by carbachol, is not affected.

4. The minimal concentration of 2,4,6-triaminopyrimidine in the bathing medium required to reach its maximal effect on the paracellular permeability is approx. 0.55 mM at pH 7.4.

5. The effect of 2,4,6-triaminopyrimidine on the paracellular permeability after carbachol stimulation is also present when 2,4,6-triaminopyrimidine is added 5 min after the addition of $1 \cdot 10^{-5}$ M carbachol.

6. 2,4,6-Triaminopyrimidine has no effect on the increases in enzyme secretion and sucrose permeability caused by $1 \cdot 10^{-8}$ M pancreozymin C octapeptide.

7. 2,4,6-Triaminopyrimidine appears in the secreted fluid at a concentration of 50% of that in the bathing medium. Upon addition of $1 \cdot 10^{-5}$ M carbachol this concentration increases up to 80%.

8. These results indicate that: (a) the increased paracellular permeability

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upon stimulation with carbachol is not caused by the enzyme secretion as such and (b) addition of 2,4,6-triaminopyrimidine prevents the carbachol-induced increase in permeability of a channel in the tight junction complex.

## Introduction

Epithelial cells are linked together by specialized structures called tight junctions, which are located close to the apex of the cells. In certain epithelia, such as rat small intestine, rabbit gall bladder, rat renal proximal tubule and frog choroid plexus, the transepithelial resistance is low, whilst in others (rat and human submandibular salivary duct, frog skin and stomach, toad and turtle urinary bladder) it is high [1]. The low electrical resistance of the leaky epithelia has in most cases, such as rabbit proximal tubule and ileum [2], been shown to be due to a high conductance of the tight junctions.

Moreno [3,4] showed that the permeability of rabbit and bullfrog gall bladder, small intestine and choroid plexus for cations, but not for anions, can be inhibited by the proton donor 2,4,6-triaminopyrimidine. This suggests the presence of separate anion and cation channels in the junctional complex. The cation channel would contain a negatively-charged group which can react with 2,4,6-triaminopyrimidine and which may play a role in cation transport [3,5].

In rabbit pancreas the permeability of the paracellular route for divalent cations [6,7] and for small neutral molecules [8] is enhanced by cholinergic compounds and by pancreozymin, substances which are known to stimulate enzyme secretion by this organ. In rabbit submandibular gland the permeability of horseradish peroxidase through the tight junctions of adjacent epithelial cells is enhanced by adrenalin [9].

The availability of 2,4,6-triaminopyrimidine as a blocker of the $Na^+$ channel in tight junctions of leaky epithelia has led us to investigate whether this channel in the rabbit pancreas is involved in the transepithelial permeability for divalent cations and neutral molecules. We have studied this in the resting state as well as after stimulation with the acetylcholine analogue, carbamylcholine, and the C-terminal octapeptide of pancreozymin.

## Materials and Methods

*Chemicals.* Carbachol, the carbamyl analogue of acetylcholine, is purchased from ACF Chemiefarm, Naarden (The Netherlands); 2,4,6-triaminopyrimidine is obtained from Sigma Chemical Company, St. Louis, MO. Pancreozymin C octapeptide is a gift from Dr. M. Ondetti (The Squibb Institute for Medical Research, Princeton, NJ).

[$^{14}$C]Urea (60 Ci/mol), [$^{14}$C]mannitol (60 Ci/mol), [$^{14}$C]sucrose (381 Ci/mol) and $^{45}CaCl_2$ (0.25—1 Ci/mol) are purchased from The Radiochemical Centre, Amersham, U.K. Pico-Fluor 15, the counting solvent, is obtained from Packard Instruments S.A. Benelux, Brussels, Belgium. All other chemicals used are commercial preparations of the highest obtainable purity.

*Methods.* The preparation of the isolated rabbit pancreas, the incubation medium, the fraction collection and the assay procedures are the same as earlier described [6,8,10].

2,4,6-Triaminopyrimidine, which has an absorption peak at 276 nm, is determined as follows: to a 25 µl sample of the bathing medium or secreted fluid is added 1 ml 10% (w/v) trichloroacetic acid solution in order to precipitate proteins. After 5 min centrifugation at 15 000 × *g* in a Phywe PH 1 centrifuge, the supernatant is diluted with 4 vols. distilled water and the 276 nm absorbance is measured in a Zeiss PMQ II spectrophotometer (final pH 1.2).

$Na^+$ and $K^+$ concentrations are determined in an Eppendorf flame photometer.

## Results

*Effects of 2,4,6-triaminopyrimidine on the paracellular permeability*

In the absence of carbachol, 10 mM 2,4,6-triaminopyrimidine has little or no effect on fluid secretion, protein secretion and sucrose permeability (Fig.

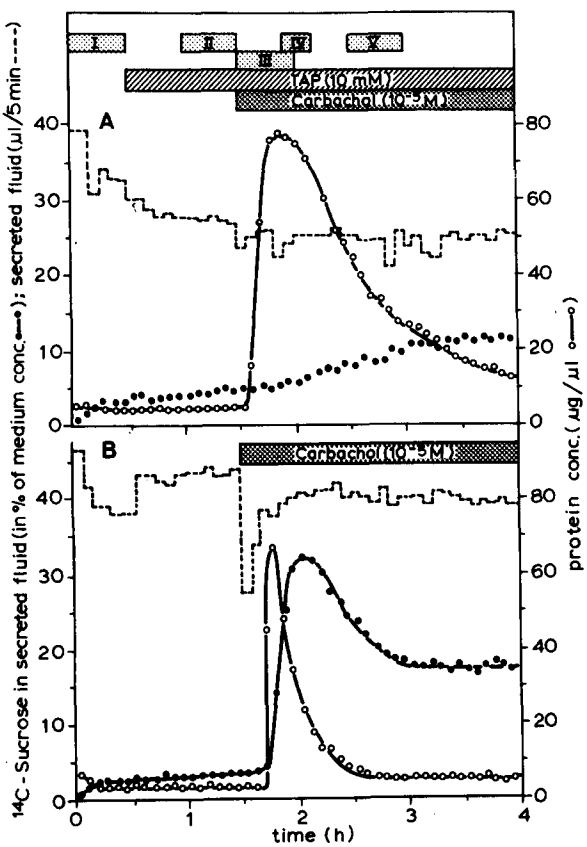


**Fig. 1. Effects of 2,4,6-triaminopyrimidine on sucrose, protein and fluid secretion by the isolated rabbit pancreas. Typical experiment showing sucrose (•——•) and protein (○——○) concentration in the secreted fluid and the rate of fluid secretion (- - - - -) before and after addition of 10 mM 2,4,6-triaminopyrimidine and $1 \cdot 10^{-5}$ M carbachol. (A) At zero time, 2 mM [$^{14}$C]sucrose; at $t$ = 0.5 h, 10 mM 2,4,6-triaminopyrimidine; and at $t$ = 1.5 h, $1 \cdot 10^{-5}$ M carbachol are added to the bathing medium. (B) Control experiment with the same conditions as (A), except that no 2,4,6-triaminopyrimidine is added. (A) and (B) are representative for seven and four experiments, respectively. Periods I—V are defined in the legend to Table I. TAP, 2,4,6-triaminopyrimidine.**

1A, first 90 min). When carbachol is present without 2,4,6-triaminopyrimidine, three effects are seen (Fig. 1B): (a) a large transient stimulation of the enzyme secretion; (b) a minor reduction in the fluid secretion and (c) an increase in the paracellular permeability for sucrose. When both carbachol and 2,4,6-triaminopyrimidine are applied (Fig 1A, 90—240 min, compared with same time period in Fig. 1B), there is little effect of 2,4,6-triaminopyrimidine on fluid secretion. However, the duration of protein secretion is extended and the permeability increase for sucrose is nearly abolished.

Table I supplies quantitative data on these effects. Carbachol is added either in $1 \cdot 10^{-5}$ M or $1 \cdot 10^{-6}$ M concentration. It appears that 2,4,6-triaminopyrimidine has little effect on fluid secretion (column A), even in the presence of carbachol (column B). The protein output after stimulation of the enzyme secretion with $1 \cdot 10^{-5}$ M carbachol is the same in the presence of 10 mM 2,4,6-triaminopyrimidine as in its absence. Stimulation with $1 \cdot 10^{-6}$ M carbachol in the presence of 10 mM 2,4,6-triaminopyrimidine produces less protein secretion (column C), whilst the latter substance alone does not affect the basal protein secretion. The most striking effects of 2,4,6-triaminopyrimidine are seen on the increased permeability for sucrose which is nearly abolished, especially after stimulation with $1 \cdot 10^{-5}$ M carbachol (columns D and E).

*Effect of varying the time of addition of 2,4,6-triaminopyrimidine*

Whereas, in the experiments described in the previous section, 2,4,6-triaminopyrimidine is always added 1 h before stimulation with carbachol, we have now investigated the effects of adding 2,4,6-triaminopyrimidine 5 min before, simultaneously with and 5 min after stimulation with $1 \cdot 10^{-5}$ M carbachol.

TABLE I

EFFECTS OF CARBACHOL AND 2,4,6-TRIAMINOPYRIMIDINE ON THE RATE OF FLUID SECRETION AND THE SUCROSE AND PROTEIN CONCENTRATIONS IN THE FLUID SECRETED BY THE ISOLATED RABBIT PANCREAS

The experiments were carried out as described for Fig. 1A. After 30 min 10 mM 2,4,6-triaminopyrimidine were added, and after 90 min carbachol ($1 \cdot 10^{-5}$ or $1 \cdot 10^{-6}$ M) was also added. The following periods can be distinguished: period I: (0—30 min) control; period II: (60—90 min) 2,4,6-triaminopyrimidine present; period III: (90—120 min) 2,4,6-triaminopyrimidine + carbachol present (protein period); period IV: (115—130 min) 2,4,6-triaminopyrimidine + carbachol present (peak period); period V: (150—180 min) 2,4,6-triaminopyrimidine + carbachol present (steady-state period). Values are given with S.E. and the number of experiments (*n*).

| Addition | Fluid secretion ratio | | Protein ratio | Sucrose ratio | | *n* |
|---|---|---|---|---|---|---|
| | A (period II/ period I) | B (period V/ period II) | C (period III/ period II) | D (period IV/ period II) | E (period V/ period II) | |
| Control | — | 0.8 ± 0.1 | 1.0 ± 0.1 | 1.3 ± 0.1 | 1.5 ± 0.1 | 4 |
| Carbachol ($1 \cdot 10^{-5}$ M) | — | 0.9 ± 0.1 | 12.6 ± 3.7 | 6.7 ± 1.4 | 5.4 ± 0.9 | 4 |
| 2,4,6-Triaminopyrimidine + carbachol ($1 \cdot 10^{-5}$ M) | 0.9 ± 0.1 | 0.8 ± 0.1 | 12.6 ± 2.0 | 1.6 ± 0.1 | 2.2 ± 0.1 | 7 |
| Carbachol ($1 \cdot 10^{-6}$ M) | — | 0.9 ± 0.1 | 5.9 ± 1.6 | 1.6 ± 0.1 | 2.1 ± 0.2 | 4 |
| 2,4,6-Triaminopyrimidine + carbachol ($1 \cdot 10^{-6}$ M) | 0.9 ± 0.1 | 0.9 ± 0.1 | 3.2 ± 0.6 | 1.4 ± 0.2 | 2.0 ± 0.1 | 3 |

TABLE II

EFFECTS OF 10 mM 2,4,6-TRIAMINOPYRIMIDINE, ADDED AT VARIOUS TIME INTERVALS BEFORE AND AFTER $1 \cdot 10^{-5}$ M CARBACHOL, ON PROTEIN AND SUCROSE SECRETION BY THE ISOLATED RABBIT PANCREAS

Values are given with S.E. and the number of experiments ($n$). Protein ratio: the amount of protein secreted in the 30 min period after carbachol addition to that secreted in the 30 min period before addition of carbachol. Sucrose ratio: ratio of the average sucrose concentration in the period 25—40 min after addition of carbachol to that before addition.

| Time interval (min) | Protein ratio | Sucrose ratio | $n$ |
|---|---|---|---|
| Control (no 2,4,6-triaminopyrimidine) | 12.6 ± 2.7 | 6.7 ± 1.4 | 4 |
| —60 | 12.6 ± 2.0 | 1.6 ± 0.1 | 7 |
| —5 | 15.4 ± 4.7 | 2.5 ± 0.1 | 3 |
| 0 | 14.5 ± 1.0 | 2.5 ± 0.4 | 3 |
| +5 | 12.3 ± 1.7 | 2.2 ± 0.3 | 3 |

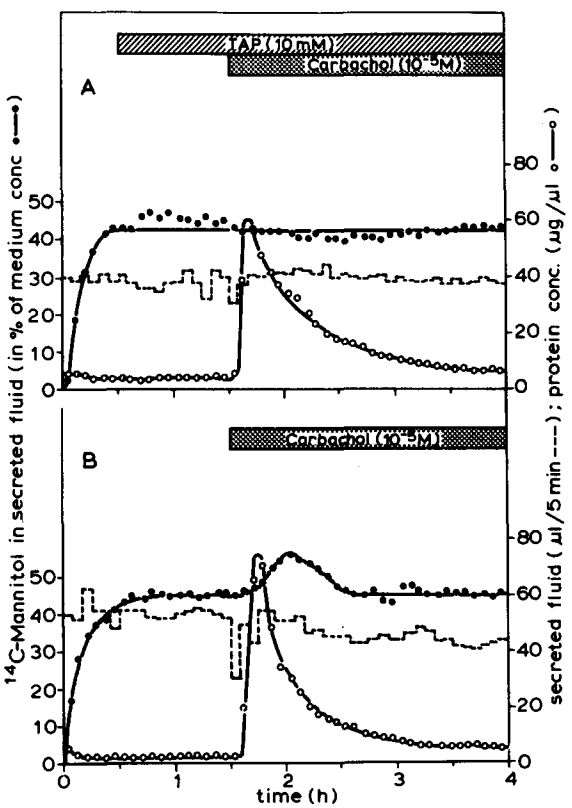


Fig. 2. Effects of 2,4,6-triaminopyrimidine on mannitol, protein and fluid secretion by the isolated rabbit pancreas. Typical experiment showing mannitol (•——•) and protein (○——○) concentration in the secreted fluid and the rate of fluid secretion (- - - - -) before and after addition of 10 mM 2,4,6-triaminopyrimidine and $1 \cdot 10^{-5}$ M carbachol. (A) At zero time, 2 mM [$^{14}$C]sucrose; at $t$ = 0.5 h, 10 mM 2,4,6-triaminopyrimidine; and at $t$ = 1.5 h, $1 \cdot 10^{-5}$ M carbachol are added to the bathing medium. (B) Control experiment with the same conditions as in (A), except that no 2,4,6-triaminopyrimidine is added. (A) and (B) are representative for three and five experiments, respectively.

In Table II the protein and sucrose concentrations are given for these experiments. It appears that 2,4,6-triaminopyrimidine inhibits the increase in sucrose permeability in all these cases, although the effects are slightly smaller. Again no effect on fluid secretion (not shown) or protein secretion is observed.

*Effects of 2,4,6-triaminopyrimidine on permeability for other non-electrolytes*

Previously, we have shown that upon addition of 2 mM urea to the bathing medium the urea reaches the same concentration in the secreted fluid as in the bathing medium and that this level is not influenced by $1 \cdot 10^{-5}$ M carbachol [8]. Addition of 2,4,6-triaminopyrimidine has no effect on the urea concentration before and after carbachol addition. However, the observed increase in the mannitol concentration in the secreted fluid just after carbachol stimulation (Fig. 2B), previously described [6,8], disappears upon addition of 2,4,6-triaminopyrimidine (Fig. 2A).

*Effects of 2,4,6-triaminopyrimidine on the permeability for calcium*

In the previous sections, the effects of 2,4,6-triaminopyrimidine on non-electrolyte secretion have been described. Since $Ca^{2+}$ is also secreted, at least in part, via the paracellular pathway [6], we have investigated the effect of 2,4,6-triaminopyrimidine on the $^{45}Ca^{2+}$ flux from bathing medium to secreted fluid.

2,4,6-Triaminopyrimidine has no effect on the basal $^{45}Ca^{2+}$ level in the secreted fluid (data not shown). When $1 \cdot 10^{-5}$ M carbachol is added simultaneously with or 5 min before or after addition of 2,4,6-triaminopyrimidine, marked inhibition of the increased $Ca^{2+}$ secretion induced by carbachol alone is noticed (Table III).

*Effects of 2,4,6-triaminopyrimidine concentration*

In all experiments described so far, we have applied 2,4,6-triaminopyrimidine in 10 mM concentration, as has been done by most other investigators. In additional experiments we have tested the effect on the sucrose permeability of lower concentrations (0.1—10 mM) of 2,4,6-triaminopyrimidine, with $1 \cdot 10^{-5}$ M carbachol added 5 min later (Fig. 3). At or above concentrations of 0.55 mM nearly maximal inhibition of sucrose permeability is found, whilst at

TABLE III

EFFECTS OF 10 mM 2,4,6-TRIAMINOPYRIMIDINE ADDED AT VARIOUS TIME INTERVALS AFTER CARBACHOL ($1 \cdot 10^{-5}$ M) STIMULATION ON PROTEIN AND $Ca^{2+}$ SECRETION BY THE ISOLATED RABBIT PANCREAS

Values are given with S.E. and the number of experiments ($n$). Protein ratio: the amount of protein secreted in the 30 min period after carbachol addition to that secreted in the 30 min period before addition of carbachol. Calcium ratio: ratio of the average $Ca^{2+}$ concentration in the period 25—40 min after addition of carbachol to that before addition.

| Time interval (min) | Protein ratio | Calcium ratio | $n$ |
|---|---|---|---|
| Control (no 2,4,6-triaminopyrimidine) | 13.0 ± 0.3 | 2.6 ± 0.5 | 3 |
| —5 | 12.7 ± 1.6 | 1.2 ± 0.1 | 3 |
| 0 | 17.7 ± 4.8 | 1.4 ± 0.1 | 5 |
| +5 | 11.1 ± 2.4 | 1.5 ± 0.2 | 5 |

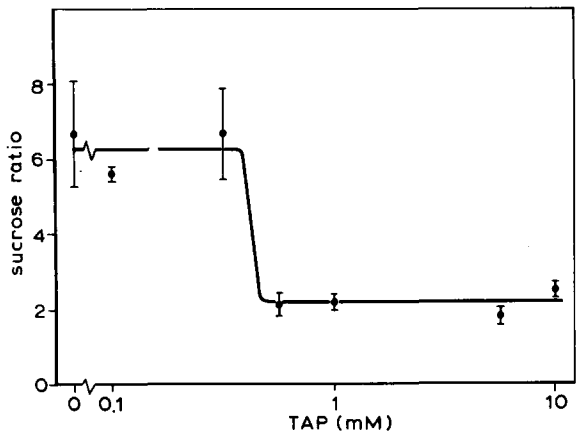


Fig. 3. Sucrose secretion after addition of $1 \cdot 10^{-5}$ M carbachol as a function of the concentration of 2,4,6-triaminopyrimidine. In all experiments 2,4,6-triaminopyrimidine is added 5 min prior to carbachol. The ordinate represents the sucrose ratio as defined in Table II. Data points represent average for three to four experiments with the S.E.

or below 0.3 mM no clear effect is shown. In these experiments the pH of the bathing medium is 7.4. The curve in Fig. 3 gives the impression of an all or none effect.

*Other effects of 2,4,6-triaminopyrimidine*

Pancreozymin or its C-terminal octapeptide ($1 \cdot 10^{-8}$ M) have approximately the same effects on enzyme secretion and sucrose permeability as $1 \cdot 10^{-5}$ M

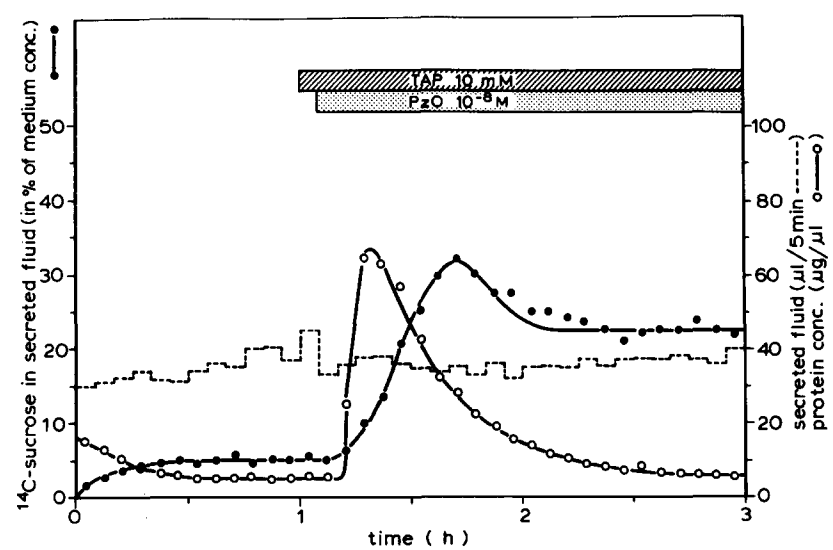


Fig. 4. Effects of 2,4,6-triaminopyrimidine and pancreozymin C octapeptide on secretion of sucrose, protein and fluid by the isolated rabbit pancreas. Typical experiment showing sucrose (•——•) and protein (o——o) concentrations in the secretory fluid and the rate of fluid secretion (------). After 60 min incubation 10 mM 2,4,6-triaminopyrimidine are added, followed after 5 min by addition of $1 \cdot 10^{-8}$ M pancreozymin C octapeptide. Representative for four experiments.

TABLE IV

EFFECT OF 2,4,6-TRIAMINOPYRIMIDINE AND CARBACHOL ON $Na^+$ AND $K^+$ CONCENTRATIONS IN THE SECRETED FLUID

The experiments were carried out as described in Fig. 1A. After 30 min, 10 mM 2,4,6-triaminopyrimidine was added, followed by addition of $1 \cdot 10^{-5}$ M carbachol at $t$ = 90 min. Period I, (0—30 min) control; period II, (60—90 min) 2,4,6-triaminopyrimidine present; period III, (120—150 min) 2,4,6-triaminopyrimidine and carbachol present. Results are expressed in mM. Values are given with S.E. and the number of experiments ($n$).

| | Period I | | Period II | | Period III | |
|---|---|---|---|---|---|---|
| | $Na^+$ | $K^+$ | $Na^+$ | $K^+$ | $Na^+$ | $K^+$ |
| Control (no drugs) | 153.0 ± 0.8 (3) | 9.6 ± 0.6 (3) | 153.2 ± 0.8 (3) | 10.3 ± 0.8 (3) | 155.4 ± 0.9 (2) | 10.1 ± 0.9 (2) |
| Experimental | 156.9 ± 2.2 (4) | 9.2 ± 0.3 (4) | 159.5 ± 3.2 (4) | 10.4 ± 0.3 (4) | 156.7 ± 1.2 (3) | 10.3 ± 0.5 (3) |

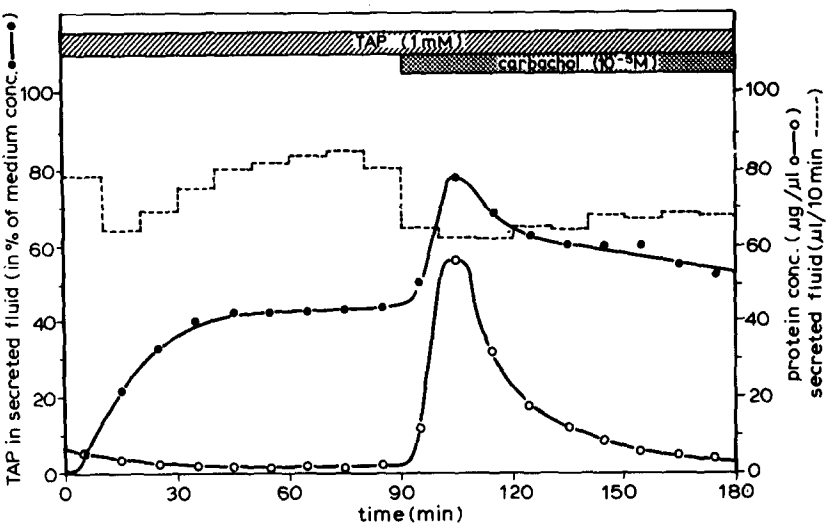


Fig. 5. Permeability of the isolated rabbit pancreas for 2,4,6-triaminopyrimidine. At zero time, 1 mM 2,4,6-triaminopyrimidine and at $t$ = 90 min, $1 \cdot 10^{-5}$ M carbachol are added to the bathing medium. Rate of fluid secretion (- - - - -) and concentrations of protein (o——o) and 2,4,6-triaminopyrimidine (•——•) are measured in the secreted fluid. Representative for four experiments.

carbachol [8]. When 10 mM 2,4,6-triaminopyrimidine is given 5 min before $1 \cdot 10^{-8}$ M pancreozymin C octapeptide, there is no inhibition of the effects of pancreozymin on the two parameters (Fig. 4). This suggests that the inhibitory effect of 2,4,6-triaminopyrimidine on the carbachol-induced increase in sucrose permeability occurs at the level of the acetylcholine receptor.

We have shown that 2,4,6-triaminopyrimidine has no effect on the $Ca^{2+}$ and sucrose concentrations in the fluid secreted by the resting pancreas. In addition, 2,4,6-triaminopyrimidine has no effect on the $Na^+$ and $K^+$ concentrations in the secreted fluid before and after carbachol addition (Table IV). The slight increase in the $K^+$ concentration was also observed in the control, where no 2,4,6-triaminopyrimidine is present. It is probably due to $K^+$ leakage from the preparation, primarily from the large amount of intestine present.

Finally, we have analyzed the appearance of 2,4,6-triaminopyrimidine in the secreted fluid after its addition to the bathing medium. Fig. 5 shows a typical experiment, in which 1 mM 2,4,6-triaminopyrimidine is added to the bathing medium at zero time. After approx. 40 min, the level of the substance in the secreted fluid equilibrated at 50% (S.E. = 1.5, $n$ = 6) of that of the bathing medium. Upon addition of $1 \cdot 10^{-5}$ M carbachol, the 2,4,6-triaminopyrimidine concentration increases to 80% (S.E. = 2.3, $n$ = 4), coincidently with the peak of the protein secretion. Thereafter, the increased 2,4,6-triaminopyrimidine concentration slowly returns to its original steady-state concentration.

## Discussion

Diamond [11] characterizes the channel through the tight junctions in the following words: 'The junctional channel is similar to the tortuous route that a microbe would follow crawling through a pile of spaghetti'. There are prob-

ably at least two different types of channels: an anion and a cation channel, as demonstrated by Moreno [3,4] who used 2,4,6-triaminopyrimidine, a proton donor which forms strong hydrogen bonds with acid groups of the cation channel. The results of Moreno for bullfrog and rabbit small intestine, gall bladder and choroid plexus have been confirmed for other leaky epithelia, viz. feline choroid plexus [12], canine ileum [13] and rabbit ileum [14,15]. For the junctional complex in the pancreatic epithelium no result has been reported so far.

In a recent paper we have reported [8] that sucrose penetrates the pancreatic epithelium entirely by the paracellular pathway, and that this route becomes more permeable after addition of pancreozymin C octapeptide and carbachol, both of which stimulate enzyme secretion. In the present paper we report the effects of 2,4,6-triaminopyrimidine on the permeability of small non-electrolytes and cations in the isolated rabbit pancreas, both before and after stimulation with carbachol.

When 2,4,6-triaminopyrimidine is added to the bathing medium without carbachol, no effect is observed in the permeabilities of $Na^+$, $K^+$, $Ca^{2+}$ and sucrose and on the rate of fluid secretion. After addition of $1 \cdot 10^{-5}$ M carbachol, a single type of effect of 2,4,6-triaminopyrimidine is observed: the normally observed permeability increases for sucrose, mannitol and $Ca^{2+}$ [6—8] are decreased. No effect on $Na^+$ and $K^+$ secretion or on enzyme secretion is noticed. The time of addition of 2,4,6-triaminopyrimidine, relative to that of carbachol (from 60 min before to 5 min after addition of carbachol), is not critical, which means that the permeability increase is a relatively slow process.

From our findings it seems unlikely that 2,4,6-triaminopyrimidine blocks the cation channel in the pancreas, as it seems to do in gall bladder and in other leaky epithelia [4,12—15]. The lack of effect of 2,4,6-triaminopyrimidine alone on the fluid secretion and on the $Na^+$ and $K^+$ concentrations in the secretory fluid all argue against such a mode of action. Although we did not carry out transepithelial potential measurements with and without 2,4,6-triaminopyrimidine, the parallelism found by Naftalin and coworkers [15—17] for rabbit ileum between the effects of this substance on fluid secretion and on $Na^+$ permeability, strongly suggests that in the rabbit pancreas 2,4,6-triaminopyrimidine has no measurable effect on the $Na^+$ permeability of the tight junction.

In the rabbit pancreas pancreozymin C octapeptide and carbachol seem to induce the permeability of another type of channel. These stimulants enhance in similar fashion the permeability of substances, which are not completely permeable in the resting state, such as sucrose, mannitol and $Ca^{2+}$, but not of monovalent ions, water and urea, which are already maximally permeable in the absence of a stimulant. So it might be that 2,4,6-triaminopyrimidine would block the permeability increase of this channel.

However, the fact that 2,4,6-triaminopyrimidine only blocks the carbachol-induced permeability increase but not that induced by pancreozymin suggests that it does not act on the channel itself but rather on the receptor level. It would act on the acetylcholine receptor but not on the pancreozymin receptor. Since atropine can block the stimulatory effects of carbachol on the enzyme secretion as well as on the tight junction permeability, it would seem that different types of acetylcholine receptors are involved in these two pro-

cesses. Alternatively, it might be that for the effect of acetylcholine on enzyme secretion, a much smaller number of acetylcholine receptors must be occupied than for its effect on tight junction permeability, and that 2,4,6-triaminopyrimidine would inhibit the interaction of acetylcholine with its receptors less efficiently than atropine. This alternative is supported by the fact that the stimulatory effect of $1 \cdot 10^{-6}$ M carbachol on enzyme secretion is also partially inhibited by 2,4,6-triaminopyrimidine.

There are further reasons to believe that the effect of 2,4,6-triaminopyrimidine in the pancreas is different to that in other epithelia. The findings of Moreno [4,18] and others [12—14] strongly suggest that only the monovalent cationic form of 2,4,6-triaminopyrimidine is the active component. It is difficult to check this for the pancreas, since lowering the pH of the bathing medium below 7.4 markedly reduces the fluid secretion, probably due to the operation of an $H^+/Na^+$ exchange system in the fluid secretion process [19]. At pH 7.4 the 2,4,6-triaminopyrimidine concentration which is just maximally effective (0.55 mM) would represent a cationic concentration of only 0.1 mM. This value is much lower than that required in other tissues. The low effective concentration, the apparent all or none effect of 2,4,6-triaminopyrimidine (Fig. 3) and its ineffectiveness on sodium permeability all suggest that the effect of 2,4,6-triaminopyrimidine in the pancreas is of a different nature than that in other leaky epithelia.

The way in which 2,4,6-triaminopyrimidine exerts its effects, thus, cannot be established with certainty, as long as we do not know the molecular explanation for the increase in transepithelial permeability by carbachol and other stimulants. We do not even know whether the effect of 2,4,6-triaminopyrimidine is due to its proton donor capacity or to another property of the molecule. Kreys et al. [13]have shown that in canine ileum 2,4,6-triaminopyrimidine also has some membrane effects such as the blocking of the unidirectional $Na^+$ flux from plasma to lumen and the absorption of several sugars. Reuss and Grady [20] have recently reported that in *Necturus* gall bladder 2,4,6-triaminopyrimidine blocks the $K^+$ conductance of the luminal membrane. This indicates that 2,4,6-triaminopyrimidine might have effects on epithelial plasma membranes other than the effects on the tight junctions.

In our previous study [8] we have obtained strong indications that the carbachol-induced increase in transepithelial permeability is not caused by the enzyme secretion as such [6]. The fact that 2,4,6-triaminopyrimidine blocks the increase in permeability without affecting enzyme secretion further supports our findings that the two effects of carbachol on the pancreas are not directly coupled.

We have also shown that 2,4,6-triaminopyrimidine crosses the epithelium from bathing medium to secreted fluid. Upon stimulation with carbachol there is an increase in the permeability for 2,4,6-triaminopyrimidine. This is not unexpected, since its molecular weight of 125 is similar to that of other substances whose permeabilities are increased. The secretion peak for 2,4,6-triaminopyrimidine does, however, coincide with the protein peak (Fig. 5), in contrast to the secretion peaks of sucrose and $Ca^{2+}$, which always occur some 10 min after the protein peak (Refs. 6 and 8, and Fig. 1 in this paper). This suggests that if 2,4,6-triaminopyrimidine passes through the tight junctions, its

transport is in some way facilitated, or else that it passes through the cells, in which case it would have to penetrate both the basal and the apical membranes.

Similar increases in permeability of junctional complexes have been observed in other epithelia. Parsons et al. [9] have shown that horseradish peroxidase can pass by way of the paracellular route between the acinar cells of the submandibular gland after stimulation with adrenalin. Linzell and Peaker [21] and Peaker and Taylor [22] have suggested that the tight junctions open up during the onset of lactation in goat and rabbit mammary gland. Simani et al. [23] have shown that cigarette smoke increases the permeability of the junctional complexes of respiratory epithelium to horseradish peroxidase. Similarly, it seems quite likely that in the pancreas the junctional complexes open up during stimulation of its exocrine secretion. The physiological significance of these phenomena is not yet clear.

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