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Immediate action of SCN^- on the apical transport of $C1^-$ in the epithelial cells of rabbit gallbladder

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Fisiologia. — *Immediate action of* SCN^- *on the apical transport of* Cl^- *in the epithelial cells of rabbit gallbladder* (*). Nota di DARIO CREMASCHI, GIULIANO MEYER, CARLO ROSSETTI, GUIDO BOTTÀ e PAOLA PALESTINI, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Il Tiocianato, posto nel lume della cistifellea di coniglio per 45 sec a varie concentrazioni (insieme col ^{36}Cl durante la misura del flusso dell'anione attraverso le membrane apicali e le giunzioni dell'epitelio), inibisce questo flusso di circa l'80% se usato in concentrazioni superiori a 17 mM circa. Riducendo i tempi di esposizione si ottiene lo stesso grado di inibizione. Si conclude che il Tiocianato opera legandosi ai siti apicali di trasporto del Cl^- inibendoli in modo pressoché immediato. Lo stiramento del tessuto aumenta la frazione insensibile al Tiocianato, mentre lascia invariata quella sensibile.

INTRODUCTION

In a previous paper we showed that SCN^- abolishes the apical entry of Cl^- into the epithelial cells of rabbit gallbladder [3]. The tissue was exposed to SCN^- for 45 min so that the doubt that the action could be indirect or metabolic was present, in spite of the fact that all results were consistent with a competition of SCN^- for Cl^- sites of transport.

The aim of this paper is to eliminate any doubt concerning this point by studying the action of the inhibitor on Cl^- influx through the apical membrane with very short times corresponding to those of the influx measurement (45 sec or less).

METHODS

New Zealand rabbits were killed by a blow on the neck. Gallbladders were excised and washed free from bile with Krebs-Henseleit solution (mM: 142.9 Na^+, 127.7 Cl^-, 24.9 HCO_3^-, 5.9 K^+, 1.2 $H_2PO_4^{2-}$, 1.2 SO_4^{2-}, 1.2 Mg^{2+}, 2.5 Ca^{2+}). Opened flat, they were mounted on a nylon mesh between two lucite chambers with the luminal surface facing upwards, exposed (0.61 cm^2) within the upper chamber, filled with 1 ml saline. After a pre-incubation period of 75 min, the tissue was bathed on the luminal side for 45 sec by the test so-

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lution added with ^{3}H-sucrose (10 μCi/ml) and ^{36}Cl (4 μCi/ml) in order to measure the Cl^- unidirectional influx into the epithelium through the apical membrane and the junctional complex. Test solutions were Krebs-Henseleit saline in which part of Cl^- was replaced by SO_4^{2-} and mannitol (control) or by SCN^- (experiment) at the reported concentrations. When SO_4^{2-} was used, mannitol was added to compensate osmolality. All salines were bubbled with 5% CO_2 + + 95% O_2; pH was 7.4 and temperature 27 °C. Analyses were performed as reported in Ref. 2.

Results

SCN^- *effects.* The action of SCN^- (present on the luminal side during the 45 sec measuring time at different concentrations) on Cl^- uptake (expressed as μeq cm^{-2} h^{-1}) is reported in fig. 1. It is to emphasize that maximal inhibition is obtained with concentrations larger than 17 mM SCN^- and that a residual Cl^- uptake, SCN^- independent (about 4 μeq cm^{-2} h^{-1}) is measured even under these conditions.

The action of the drug, used with a maximal concentration (25 mM), was also tested at different exposure times (30 and 45 sec). When a 30 sec period was employed, the flux measuring time was also reduced to this time (the mea-

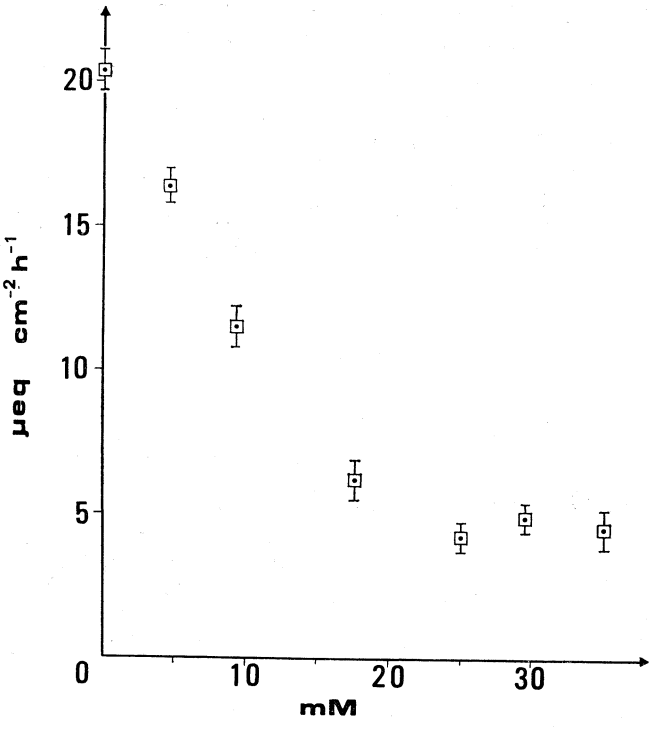

Fig. 1. - SCN^- effects on Cl^- influx. Abscissa: SCN^- concentrations (mM); Ordinata: Cl^- influx (μeq cm^{-2} h^{-1}). Resulted are reported as mean ± standard error. (7 experiments per point).

sure of the unidirectional influx is constant if taken between 0 and 75 sec, see Ref. 3). The result is reported in Table I: the rate of Cl^- entry into the epithelium is inhibited to the same extent after both 30 and 45 sec of treatment.

Stretch effects. If the gallbladder, opened flat, is stretched during mounting, Cl^- influx increases significantly from 21.9 to 28.0 μeq cm^{-2} h^{-1} (Table II).

TABLE I

Cl^- influxes (lumen to epithelium) at different times of measurement with different times of exposure to SCN^-.

In controls only $^{36}Cl^-$ was present in the luminal bath. In the esperiments $^{36}Cl^-$ and SCN^- (25 mM) were simultaneously present in the luminal bath for 30 or 45 sec. Results are reported as means ± standard error with the number of experiments in parenthesis. P = statistical probability.

Exposure time (sec) to $^{36}Cl^-$ and SCN^-	Cl^- influx (μeq cm^{-2} h^{-1})		P
	Control	SCN^-	
30	20.6 ± 2.5 (5)	4.4 ± 1.4 (6)	< 0.01
45	21.0 ± 1.2 (5)	4.0 ± 0.8 (6)	< 0.01
P	n. s.	n. s.	

TABLE II

SCN^--dependent and SCN^--independent Cl^- influxes (lumen to epithelium) measured under different stretch conditions of the tissue.

Results are reported as means ± standard error with the number of experiments or freedom degrees (*fd*) in parenthesis. P = statistical probability.

	Unstretched conditions Cl^- influx (μeq cm^{-2} h^{-1})	Stretched conditions Cl^- influx (μeq cm^{-2} h^{-1})	P
Control	21.9 ± 1.1 (12)	28.0 ± 2.5 (12)	< 0.05
SCN^- present . . .	4.6 ± 0.4 (23)	9.7 ± 1.4 (13)	< 0.01
Δ	17.3 ± 1.0 (*fd* = 33)	18.3 ± 2.8 (*fd* = 23)	n. s.

Correspondingly, in the presence of 25 mM SCN^-, the residual Cl^- influx is again significantly increased under the stretched condition (4.6 vs. 9.7 μeq $cm^{-2} h^{-1}$, see Table II), whereas the SCN^--dependent fraction, calculated as a difference between the respective control and the residual Cl^- influx, is statistically equal (17.7 and 18.3 μeq $cm^{-2} h^{-1}$).

Discussion

The fact that with 30 or 45 sec of exposure to SCN^- the same extent of inhibition is obtained points out that the maximal action of the drug is immediate or takes place with a lag negligible with respect to the measuring time (i.e. very few sec). This result, combined with the fact that the exposure to SCN^- is only luminal, confirms the previous [3] conclusion that the inhibitor acts directly on the transport sites of Cl^-, competing for them.

In the apical membrane no Cl^- conductance exists [4, 6] so that the entry of the anion is only neutral and mediated by a co-transport with Na^+ [2, 6]; thus, SCN^- should bind to the Cl^- sites of this co-transport. The fraction of Cl^- influx which turns out to be SCN^--insensitive should be related to the slight Cl^- conductance of the junctional complex [1]. Stretch manoeuvres, which should affect paracellular pathways [1, 5], do not modify the SCN^--sensitive component of Cl^- influx, whereas the SCN^--insensitive fraction largely increases.

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