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RENAL EXCRETION OF STEVIOSIDE IN RATS

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ABSTRACT.—The renal excretion of stevioside, a glycoside extracted from the leaves of *Stevia rebaudiana*, and its effect on renal excretion of several substances, was studied through clearance techniques in Wistar rats. After a control period, stevioside was infused iv at four concentrations (4, 8, 12, and 16 mg/kg). During all the experiments no significant changes in inulin clearance (C_{In}) were observed. The stevioside infusion induced a significant increase in the *p*-aminohippuric acid clearance (C_{PAH}), fractional sodium excretion ($FeNa^+$), urinary flow as percent of glomerular filtration rate (V/GFR), and glucose clearance (C_G) when compared to controls, but these effects were absent with the dose of 4 mg/kg. The stevioside clearance (C_S) was higher than the C_{In} and lower than the C_{PAH} at all the doses employed in this study. These results indicate that the stevioside is secreted by renal tubular ephithelium and induces diuresis and natriuresis and a fall in renal tubular reabsorption of glucose.

Stevia rebaudiana Bertoni (Asteraceae), a sweet herb indigenous to Paraguay, has been the subject of scientific interest for half a century because of the sweetness of its leaves. Stevioside is the major sweet *ent*-kaurene glycoside constituent of *S. rebaudiana*, representing approximately 3–8% of the dried leaves. It is formed by three glucose molecules and steviol, a diterpenic carboxylic alcohol (1).

Both experimental and human use of pure stevioside or *S. rebaudiana* extracts made it clear that some effects on cardiovascular parameters can be detected (2–4). We have shown that purified stevioside in rats induces hypotension, diuresis, and natriuresis, these effects being probably dependent on prostaglandin activity (5).

The present experiment was undertaken to evaluate the renal excretion of stevioside and clarify the actual participation of this compound on the renal excretion of some substances in rats, since it has been previously suggested that the stevioside induces an inhibitory effect on monosaccharide transport in the intact rat liver (6). Since stevioside is known to produce diuresis and natriuresis it is important to study its own renal excretion mechanism.

RESULTS AND DISCUSSION

Table 1 summarizes the results in rats

TABLE 1. Effect of Infusion of Stevioside (4, 8, 12, and 16 $mg \cdot kg^{-1} \cdot h^{-1}$) on Clearance of Different Substances in Rats.[a]

DT (mg/kg)	C_{in} ($ml.min^{-1} \cdot kg^{-1}$)	C_{PAH} ($ml.min^{-1} \cdot kg^{-1}$)	C_S ($ml.min^{-1} \cdot kg^{-1}$)	C_G ($ml.min^{-1} \cdot kg^{-1}$)	FeNa+ (%)	V/GFR (%)
4						
(C) . . .	6.66 ± 0.30	12.88 ± 1.02	0.00	0.0	0.61 ± 0.07	1.41 ± 0.08
(S) . . .	6.02 ± 0.43	12.73 ± 1.11	7.21 ± 1.41**	0.0	0.79 ± 0.06	1.56 ± 0.11
8						
(C) . . .	6.33 ± 0.35	15.60 ± 0.98	0.00	0.0	0.58 ± 0.05	1.47 ± 0.07
(S) . . .	6.51 ± 0.66	19.20 ± 1.13*	9.63 ± 1.04**	1.33**	1.12 ± 0.11**	2.19 ± 0.07**
12						
(C) . . .	5.69 ± 1.05	15.38 ± 1.42	0.00	0.00	0.70 ± 0.10	1.87 ± 0.24
(S) . . .	5.92 ± 0.56	28.30 ± 2.84**	10.23 ± 1.78**	1.84**	1.53 ± 0.15**	2.67 ± 0.22*
16						
(C) . . .	6.29 ± 0.71	16.70 ± 3.76	0.00	0.00	0.60 ± 0.04	1.39 ± 0.10
(S) . . .	6.30 ± 0.57	33.40 ± 2.55**	12.50 ± 2.10**	2.38**	1.55 ± 0.20**	2.36 ± 0.11**

[a]Abbreviations: DT, dose and time period; C, control period; S, stevioside period; C_{in}, inulin clearance; C_{PAH}, *p*-aminohippuric acid clearance; FeNa+, fractional urinary sodium excretion; C_S, stevioside clearance; C_G, glucose clearance; V/GFR, urine flow as percentage of glomerular filtration rate. Significant relative to control (C) values: *$p<0.05$, **$p<0.01$; $n = 40$.

of the measurements of clearances of a number of substances in control and stevioside-treated rats at four concentrations (4, 8, 12, 16 $mg \cdot kg^{-1} \cdot h^{-1}$).

The *p*-aminohippuric acid clearance (C_{PAH}) values, on estimation of renal plasma flow, increased significantly at the concentrations of stevioside used, but this effect was absent with the dose of 4 $mg \cdot kg^{-1} \cdot h^{-1}$. Despite the significant increase in C_{PAH} induced by stevioside, glomerular filtration rate, measured by inulin clearance (C_{in}), did not change in our experiments, a fact that may be explained by vasodilation of both the afferent and efferent arterioles. This finding is in agreement with other studies in which vasodilating substances like verapamil (11,12) and secretin or acetylcholine (13) were administered to rats. A decrease in total renal vascular resistance has been proposed to explain the renal vasodilation promoted by stevioside (14).

Previous studies have indicated that intrarenal and iv infusion of a vasodilator is normally associated with an increase in urinary sodium excretion (15). As shown in Table 1, fractional urinary sodium excretion ($FeNa^+$) and urinary flow based on percentage of glomerular filtration rate (V/GFR) increased in three groups after stevioside infusion, although the increase was more pronounced in the animals receiving the higher doses of stevioside. The results can be explained as follows. The fact that H_2O and sodium excretion increased following stevioside infusion in spite of an unchanged glomerular filtration rate may be explained by decreased H_2O and sodium reabsorption in the proximal tubules. The greater enhancement of renal blood flow results in more marked changes in the Starling forces around the proximal convolution, resulting in a substantial decrease of the proximal tubular reabsorption and an increase in urinary flow and sodium excretion. Stevioside may act directly on the mechanism of tubular sodium reabsorption. Therefore, the experimental model used in the present study is not suitable for discriminating between vascular and tubular effects.

It can be seen (Table 1) that stevioside at all doses employed, except the lowest, induced a statistically significant increase in glucose clearance (C_G) when compared to controls and exhibited a dose-dependent effect. These data are in agreement with previous evidence that stevioside and other *S. rebaudiana* natural products exert an inhibitory effect on monosaccharide transport in the intact rat liver (6). This effect presumably occurs at the level of the cell membrane, inhibiting the glucose transport system.

An early study (16) showed that the oral administration of stevioside to a rooster was associated with intact excretion and reasonable intestinal absorption of this product. This finding was not confirmed in subsequent work by other authors (17), who reported the possibility of the stevioside being degraded to the aglycone steviol and the three glucose molecules by rat intestinal microflora in vitro. In the present study, it was not possible to correlate directly the results of C_G with the excretion of the intact stevioside, since steviol was not found in either the plasma or urine.

Another point that deserves to be mentioned is the fact that the mean value of the stevioside clearance (C_S), at all doses employed, is higher than the C_{in} and lower than the C_{PAH} (Table 1). Renal excretion of inulin is carried out by glomerular filtration. Renal excretion of PAH is the result of glomerular filtration and tubular secretion, although there is a small amount of tubular reabsorption (18). Also, some PAH may be protein-bound and/or metabolized. It seems possible that at least part of the excretion of stevioside in the urine is due to a secretory mechanism at the level of the renal tubular ephithelium.

EXPERIMENTAL

Samples of *S. rebaudiana* were collected in

Paraguay. Stevioside was extracted and purified from dried *S. rebaudiana* leaves (identified by Dr. Antonio Barioni Gusman, University of São Paulo) as described previously (7).

Forty normal male Wistar rats, weighing an average of 345 g, with free access to food and drinking H_2O were used. Animals were anesthetized ip with 30 mg/kg of sodium pentobarbital, and placed on a heated table, and a tracheostomy was performed. One jugular vein was catheterized for administration of priming doses and sustained infusion of inulin and *p*-aminohippuric acid (PAH) according to classical clearance measurement techniques (8,9). Isotonic Ringer's solution containing 2% PAH and 10% inulin was infused at the rate of 0.03 ml/min throughout the course of the experiment. Stevioside was infused iv through another catheterized jugular vein, which was not used during the control period. One carotid artery was cannulated for collection of blood samples. A catheter was introduced into the urinary bladder for timed urine collection. The animals were distributed in four groups of ten animals each; according to the priming and infusion doses of 4, 8, 12, and 16 $mg \cdot kg^{-1} \cdot h^{-1}$. The experiments were divided into two periods of 30 min, one a control period, and the other a period of infusion of different stevioside concentrations. Isotonic Ringer's solution containing 3% of stevioside was used. In the control and stevioside infusion periods, the animals received a similar perfused volume per g of body wt.

Inulin concentration in plasma and urine was determined by the anthrone method (8). Plasma and urinary PAH concentrations were measured by colorimetry (5). The sodium concentrations in urine and plasma were determined through a Klina flame photometer (Beckman Instruments). Glucose and stevioside detections and determinations in plasma and urine were obtained by 2D tlc, elution and colorimetric estimations by anthrone reagent (10). The Tukey test was used for statistical analysis of the data, and the results are presented as the means ± SEM, with the critical level of significance set at $P < 0.05$.

LITERATURE CITED

1. E. Mosettig, V. Beglinger, F. Dolder, H. Licht, P. Quitti, and J.A. Waters, *J. Am. Chem. Soc.*, **85**, 2305 (1963).
2. G.A. Von Schmeling, *Bol. Sanat. Sao Lucas*, **94**, 67 (1967).
3. G. Humboldt and E.M. Boeck, *Arq. Bras. Cardiol.*, **30**, 275 (1977).
4. E.M. Boeck and G. Humboldt, *Cienc. Cult. (Sao Paulo)*, **33**, 208 (1981).
5. M.S. Melis, R.E. Maciel, and A.R. Sainati, *IRCS Med. Sci.*, 1230 (1985).
6. E.L. Ishii, A.J. Schwab, and A. Bracht, *Biochem. Pharmacol.*, **36**, 1417 (1987).
7. M. Alvarez, A. Bracht, and E.L. Ishii, *Arq. Biol. Tecnol.*, **24**, 179 (1981).
8. J. Fuhr, J. Kaczmarczyk, and C.D. Kruttgen, *Klin. Wochenschr.*, **33**, 729 (1955).
9. H.W. Smith, N. Finkelstein, L. Alminosa, B. Crawford, and M. Graber, *J. Clin. Invest.*, **24**, 338 (1945).
10. J. Metiver and A.M. Viana, *J. Exp. Bot.*, **30**, 805 (1979).
11. M. MacLaughlin, M. Mello Aires, and G. Malnic, *Arq. Bras. Cardiol.*, **32**, 335 (1979).
12. M.S. Melis and R.E. Maciel, *Cienc. Cult. (Sao Paulo)*, **38**, 154 (1986).
13. N. Lameire, R. Vanholder, S. Ringoir, and I. Leusen, *Circ. Res.*, **47**, 839 (1980).
14. M.S. Melis and A.R. Sainati, *J. Ethnopharmacol.*, in press.
15. L.E. Earley and R.M. Friedler, *J. Clin. Invest.*, **45**, 542 (1966).
16. M. Pomaret and R. Lavielle, *Bull. Soc. Chim. Biol.*, **13**, 1248 (1931).
17. R.E. Wingard, J.P. Brown Jr, F.E. Enderlin, J.A. Dale, R.L. Hale, and C.T. Seitz, *Experientia*, **36**, 519 (1980).
18. W.B. Kinter, *Am. J. Physiol.*, **196**, 1141 (1959).

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