ACKNOWLEDGMENT The authors are grateful to Dr G S Pahwa, Scientist, for his help in the statistical evaluation of the data.

REFERENCES

1 Sastry BN. *The wealth of India*. New Delhi: Council of Scientific and Industrial Research 1952; **3** : 167
2 Atal CK, Siddiqui MA, Zutshi U, *et al*. Non-narcotic orally effective, centrally acting analgesic from an ayurvedic drug. *J Ethnopharmacol* 1984; **11** : 309
3 Zutshi U, Sharma SC, Kaul JL, Atal CK. Kinetic fate of potassium embelate, a non-narcotic centrally acting analgesic after oral and intravenous administration. *Pharmacology* 1990; **40** : 1
4 Booth RFG, Clark JB. A rapid method for the preparation of relatively pure metabolically competent synaptosomes from rat brain. *Biochem J* 1978; **176** : 365
5 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193** : 265

中国药理学报 *Acta Pharmacologica Sinica* 1991 May; **12** (3) : 219–222

Effects of estradiol on carbonic anhydrase and Mg^{2+}–HCO_3^-–ATPase activities in rat duodenal microvilli and kidney tubules

CHEN Hui[1], SUZUKI Shiro (*Department of Pharmacology, Kanazawa Medical University, Uchinada, Ishikawa 920–02, Japan*)

ABSTRACT The cytosol carbonic anhydrase (CA) and microvillus membrane Mg^{2+}-dependent, HCO_3^--stimulated ATPase (Mg^{2+}–HCO_3^-–ATPase) activities implicated in ion transport were determined in duodenal mucosa and renal tubule of ovariectomized and estradiol (E_2)-treated ovariectomized rats. CA and Mg^{2+}–HCO_3^-–ATPase activities in duodenum remained unchanged after ovariectomy, and sc E_2 200 $\mu g \cdot d^{-1} \times 7$ d decreased the activity of CA. Both the enzymes in kidney exhibited a similar sensitivity to ovariectomy, and the lowered activity of Mg^{2+}–HCO_3^-–ATPase following ovariectomy was restored to near normal after administration of E_2. These results suggest that E_2 may be a factor in regulation of the above enzymes from the duodenum and kidney of rats, the regulation of E_2 on these 2 enzymes in rat duodenum is greatly different from that in rat kidney.

KEY WORDS estradiol; carbonic anhydrase; magnesium adenosine triphosphatase; microvilli; duodenum; kidney tubules

Received 1989 Jun 23 Accepted 1991 Mar 15

[1] Now in *Department of Clinical Pharmacology, Tongji Medical University, Hankou 430030, China*

Estrogen receptors have been described in a number of nonreproductive target tissues, including kidney, liver, gut, heart and brain. The induction of estrogen on some enzyme systems, for example, 16-hydroxysteroid dehydrogenase, carbonic anhydrase and Na^+–K^+–ATPase was reported in various tissues. CA in the cytosol and Mg^{2+}–HCO_3^-–ATPase in the brush border membrane of duodenal mucosa and renal tubule are thought to be related to several ionic transports across the membrane, yet little is known of the biochemical effect of estrogen on these enzymes in duodenum and kidney. In this paper, both CA and Mg^{2+}–HCO_3^-–ATPase activities of duodenal mucosa and renal tubule from ovariectomized and E_2-treated ovariectomized rats were examined to clarify the effect of estrogen in the enzyme level.

MATERIALS AND METHODS

Rats Wistar ♀ rats, weighing 196 ± SD 10 g, were used. Bilateral ovariectomies

were performed dorsally 3 wk prior to the experiment, E_2-treated rats were given sc E_2 200 $\mu g \cdot d^{-1} \times 7$ d started at 2 wk after ovariectomy. All rats were fed a standard rat-chow (Dviental Co, Japan) and maintained on tap water *ad lib* at room temperature (23 ± 2℃) with a 12-h light and 12-h darkness cycle.

Chemicals Disodium adenosine-5′-triphosphate (Na_2-ATP) was obtained from Boehringer (FRG), estradiol benzoate from Merck-Banyu Co (Japan). Other chemicals were all reagent grade.

Sample preparation Rats were under pentobarbital anesthesia. The entire intestine was perfused via the portal vein and abdominal aorta with cold heparin-saline to minimize the tissue contamination by carbonic anhydrase-rich erythrocytes. The duodenal portion, approximately 12 cm in length, was excised from the pylorus. The right kidney was decapsulated and pieces of cortex were used as kidney samples. The duodenal mucosa was scraped out with a glass slide and weighed. The crude brush border membranes were prepared by calcium precipitation procedure similar to that reported[1,2]. All manipulations were carried out at 0-4℃, and all samples were stored at -20℃ until use.

Enzyme assay CA activity was measured by colorimetric method[3]. Mg^{2+}-HCO_3^--ATPase activity was assayed in a manner described by Suzuki *et al*[4]. The released inorganic phosphate (P_i) was determined colorimetrically[5]. Protein content was determined by Folin-phenol method using crystalline bovine serum albumin (Sigma) as standard. All of the results were given as $\bar{x} \pm$ SD. The P values were calculated by t test.

RESULTS

Effect on CA activity Changes in the cytosol CA activity of duodenal mucosa and kidney tubule are shown in Fig 1. The activity of CA in duodenum did not change until 2 wk after ovariectomy, and administration of E_2 200 $\mu g \cdot d^{-1} \times 7$ d to ovariectomized rats lowered the specific activity of this enzyme. Ovariectomy markedly inhibited renal CA activity, but there was no significant change on this enzyme activity in ovariectomized rats after treatment with E_2.

Effect on Mg^{2+}-HCO_3^--ATPase activity Mg^{2+}-HCO_3^--ATPase activity in brush border fraction of duodenal mucosa was not significantly altered by ovariectomy, and E_2 administered to ovariectomized rats had no influence on this ATPase. The specific activity of Mg^{2+}-HCO_3^--ATPase in kidney microvillus membrane was decreased at 2 wk after ovariectomy, and replacement with E_2 in a dose of 200 μg on 7 consecutive days restored the lowered enzymatic activity to control levels (Fig 1).

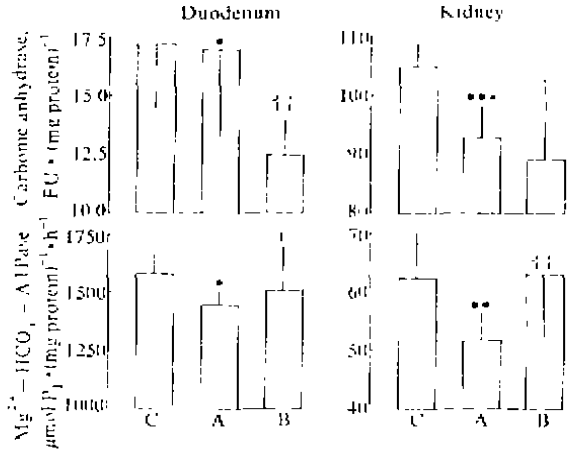

Fig 1. Effects of ovariectomy and sc estradiol (E_2) (200 $\mu g \cdot d^{-1} \times 7$ d) on CA and Mg^{2+}-HCO_3^--ATPase activities in duodenum and kidney of rats. A) ovariectomized group. B) E_2-treated ovariectomized group. $n = 6$, $\bar{x} \pm$ SD. $^{*}P > 0.05$, $^{**}P < 0.05$, $^{***}P < 0.01$ *vs* control (C). $^{+}P > 0.05$, $^{++}P < 0.05$ *vs* ovariectomized group.

DISCUSSION

CA is a zinc metalloenzyme which catalyzes the reversible hydration of carbon dioxide, and may be related to the membrane transport of protons and bicarbonate ions in organs of secretion. Ths present studies confirmed the depressing effect of E_2 on CA activity in duodenal mucosa of rats. This finding suggests that duodenal CA is a target enzyme of E_2 action and the effect of E_2 acts through inhibiting synthesis of the enzyme protein.

The biochemical basis of the effect of estrogen is not yet understood. It has been reported that the treatment with acetazolamide, a specific inhibitor of the CA, induced a diminution of acid secretion[6] and an improvement of gastric microcirculation by enhancing local blood flow[7]. The estrogen treatment was associated with a decreased morbidity of peptic ulcer[8]. It was postulated that E_2 induced a decrease of CA activity by lowering enzyme synthesis, and the effect of E_2, therefore, may act as a factor in the protection of duodenal mucosa. In the present experiment, it was not shown that the CA activity of rat kidney was affected by E_2 replacement.

Duodenal Mg^{2+}–HCO_3^-–ATPase is thought to be implicated in the process of HCO_3^- transport. The present work indicated that the duodenal ATPase was independent of E_2 treatment.

In this study, we also observed E_2–induced increase in Mg^{2+}–HCO_3^-–ATPase activity of rat kidney. The above activation of the ATPase by E_2 may be due to enzyme protein synthesis, and suggests a role of this hormone in maintaining the ATPase activity which is involved in HCO_3^- reabsorption and H^+ secretion in rat renal tubule. With respect to the functional significance of estrogen in kidney, it has been reported that the E_2–induced antinatriuresis is mediated by Na^+–K^+– ATPase of rat renal cortex[9]. However, there was no sufficient evidence to demonstrate the action of estrogen in kidney function.

These results suggest that E_2 may play a role in the regulation of the activities of CA and Mg^{2+}–HCO_3^-–ATPase from rat duodenum and kidney, the sensitivity of duodenum and kidney to the action of E_2 on the above enzyme activities is different.

REFERENCES

1 Schmitz J, Preiser H, Maestracci D, Ghosh BK, Cerda JJ, Crane RK. Purification of the human intestinal brush border membrane. *Biochim Biophys Acta* 1973; **323**: 98

2 Malathi P, Preiser H, Fairclough P, Mallett P, Crane RK. A rapid method for the isolation of kidney brush border membranes. *Biochim Biophys Acta* 1979; **554**: 259

3 Suzuki S. The presence and properties of Mg^{2+}–HCO_3^-–stimulated and SCN^-–inhibited ATPase in mouse kidney and some relationships between ATPase and carbonic anhydrase. *Comp Biochem Physiol* 1978; **59B**: 27

4 Suzuki S, Takamura S, Yoshida J, Ozaki N. Effect of aldosterone antagonists on aldosterone–induced activation of Mg^{2+}–HCO_3^-–ATPase and carbonic anhydrase in rat intestinal mucosa. *J Steroid Biochem* 1985; **23**: 57

5 Nakamura M. Colorimetric determination of phosphorus. *Nippon Nogei Kagaku Kaishi* 1950; **24**: 1

6 Puşcaş I. Treatment of gastroduodenal ulcers with carbonic anhydrase inhibitors. *Ann NY Acad Sci* 1984; **429**: 587

7 Barbarino F, Togônel E, Brilinschi C. The effects of acetazolamide and of ethoxaolamide on gastric microcirculation in rats (histochemical study). *Ann NY Acad Sci* 1984; **429**: 601

8 Everson RB, Byar DP, Bischoff AJ. Estrogen predisposes to cholecystectomy but not to stones. *Gastroenterology* 1982; **82**: 4

9 Berns J, Browning G, Douglas J. Estradiol (E_2) induced changes in renal cortical Na^+–K^+–ATPase. *Kidney Int* 1984; **25**: 325

雌二醇对大鼠十二指肠粘膜和肾小管碳酸酐酶及 Mg^{2+}–HCO_3^-–ATP 酶活性的影响

陈　汇[1]、铃木史郎
(金沢医科大学药理学教研室，石川 920–02，日本)

提要　测定双侧卵巢切除后 sc 雌二醇(200 μg · d^{-1} × 7 d)替代的大鼠十二指肠粘膜和肾小管细胞碳酸酐酶及刷状缘膜中 Mg^{2+}–HCO_3^-–ATP 酶活性．结果表明，卵巢切除对十二指肠的酶活性无明显影响，且雌二醇可抑制碳酸酐酶活性；在肾脏，这两种酶均对性腺切除敏感，且雌二醇诱导了 Mg^{2+}–HCO_3^-–ATP 酶活性．提示雌激素对肠道及肾脏酶活性的调节起到一定的生理作用，并且这种调节作用在肠道及肾脏是有差异的．

关键词　雌二醇；碳酸酐酶；镁三磷酸腺苷酶；微绒毛；十二指肠；肾小管

中国药理学报　*Acta Pharmacologica Sinica*　1991　May;　**12**　(3)：　222–225

在治疗妊娠高血压时硫酸镁的药动学–药效学模型

陈　刚、卢建丰、曹　文　(南京军区总医院，南京 210002，中国)

Pharmacokinetic–pharmacodynamic model of magnesium sulfate in treatment of pregnancy–induced hypertension

CHEN Gang，LU Jian–Feng，CAO Wen
(*General Hospital of Nanjing Armed Forces，Nanjing 210002，China*)

ABSTRACT　Ten pregnancy–induced hypertension gravidas aged 24–36 (28.1 ± 3.31) yr were infused iv with fast (7.5 g in 1 h) and slow (7.5 g in 5 h) rates consecutively．Blood pressure were measured and blood samples were collected at 0，1，6，7，9，11，15 h．The drug concentration of each sample in the serum was determined by colorimetric method．The integrated pharmacokinetic and pharmacodynamic model was used to analyze the data．The serum data were fitted to a biexponential equation and the effect data were fitted to a sigmoid E_{max} model．Their pharmacodynamic parameters were: k_{e0} = 0.52 · h^{-1}，$T_{\frac{1}{2}(k_{e0})}$ = 1.33 h，E_{max} = 20.5%，$C_e(50)$ = 6.15 μg · ml^{-1}，γ = 3.64．k_{e0} was not equal to k_{21} of the mammillary model．It shows that the site of action is situated in the effect compartment．The predicted effect could last 24 h．

KEY WORDS　hypertension；magnesium sulfate；intravenous infusions；pharmacokinetics；blood pressure

提要　10 名年龄 24–36 岁妊娠诱发高血压的孕妇，快 (7.5 g in 1 h) 慢 (7.5 g in 5 h) iv 连续滴注后，在 0，1，6，7，9，11 和 15 h 取血，测定血药浓度与血压．以 PK–PD 结合模型分析数据，血药浓度–时间曲线符合二房室模型，药效–时间曲线符合 Sigmoid E_{max} 模型．其参数：k_{e0} = 0.52 (h^{-1})，$T_{\frac{1}{2}(k_{e0})}$ = 1.33 h，E_{max} = 20.5%，$C_e(50)$ = 6.15 μg · ml^{-1}，γ = 3.64．当 $k_{e0} \neq k_{21}$ 时表示作用部位可能在效应室，预测药效将维持 24 h．

关键词　高血压；硫酸镁；静脉内输注；药物动力学；血压

Received 1989 Nov 23　　Accepted 1990 Dec 2

临床上常 iv 滴注硫酸镁注射液治疗妊娠高血压症．关于它的药动学特性[1]、药效学[2]、与给药方案[3]的研究都已有报道．然而 Mg^{2+} 血清浓度与药效呈不平行升降现象的研究尚未见报导．Sheiner 等[4]提出的药动学–药效学结合模型，对此研究提供了有力的工具．本文拟尝试以结合模型技术，分析硫酸镁血药浓度–效应的实验数据，以预测最大药效、药效滞后于血药浓度的时间和药效可能维持的时间．