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Interaction of Furosemide and Phenytoin in the Rat (42346)

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Abstract. The interaction of phenytoin and furosemide was examined in rats. After 2 or more weeks of pretreatment with phenytoin, the diuretic activity of furosemide given orally was decreased. This was due primarily to a generalized decrease in absorption from the gastrointestinal tract. Following intravenous administration of furosemide to phenytoin pretreated rats, a decrease in diuretic activity also occurred. Excretion of furosemide was not altered. Thus, phenytoin pretreatment would also appear to interfere directly with the renal action of furosemide. © 1986 Society for Experimental Biology and Medicine.

An interaction between furosemide and phenytoin was first reported by Ahmad in 1974 (1). He compared the effect of furosemide in epileptic patients being treated with phenytoin with the response of the diuretic in normal staff members. The diuretic response in the patients was smaller and delayed in onset after oral adminstration. A lesser response was also seen after iv administration. He hypothesized that phenytoin decreased renal responsiveness to furosemide and also delayed absorption of furosemide from the GI tract in his epileptic patients. Renal interference by phenytoin was based on the observation that furosemide produced less diuresis after iv administration in the presence of the anticonvulsant. The decrease in absorption of furosemide caused by phenytoin was based on a delayed onset of the action of the diuretic when given orally in the presence of the anticonvulsant. In a study by Fine and co-workers (2) in which normal subjects were used, prior administration of phenytoin was found to result in lower plasma levels of furosemide. They surmised that there was no renal interaction, believing that the decrease in the plasma level of furosemide was sufficient to explain the lesser renal action.

The objectives of the present study were to determine if phenytoin affected the activity of furosemide in a laboratory animal such as the rat and, if so, then to determine if absorption from the GI tract and/or renal interference were involved in the interaction.

Methods. Male Sprague–Dawley rats, 150–160 g, were treated with phenytoin, 50 mg/kg, intraperitoneally, daily, for varying periods of time. Sodium phenytoin was dissolved in a vehicle of alkaline saline (0.025 *N*).

Diuretic activity was determined as follows. Food, but not water, was removed the night before the assay. At the beginning of the assay, a load of isotonic sodium chloride solution 50 ml/kg, was administered orally or intraperitoneally. Furosemide was dissolved in the load. Rats were placed individually in metabolism cages and urine was collected for 3 hr. Excreted volumes were recorded each hour and at the time required to excrete 50% of the volume of the load. Gentle pressure was applied to the bladder to obtain more complete emptying. Sodium concentrations were determined using an Instrumentation Laboratory flame photometer, Model 343.

Biological samples were analyzed for furosemide using a modification of method II of the HPLC assay of Lin *et al.* (3). They used sodium phenobarbital as an internal standard. This was monitored at 254 nm while furosemide was measured at 280 nm. Thus, a dual-channel ultraviolet detector was required. In the assay here, ethacrynic acid was used as the internal standard, since it could also be monitored at 280 nm, thus permitting the use of only a single-channel ultraviolet detector. The procedure was as follows: To 0.2 ml of urine or plasma, 20 μl of internal standard (ethacrynic acid in methanol, 1 mg/ml) and 0.4 ml of acetonitrile were added. This was mixed and centrifuged to precipitate the protein. The supernatant was reduced to a volume of about 150 μl by evaporation under a nitrogen atmosphere at room temperature. Samples of 5–25 μl were used for HPLC, with settings of

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0.01 AUFS at 280 nm and a flow rate of 2 ml/min using a mobile phase of acetonitrile: 0.01 *M* sodium acetate (25:75). Retention times were 5 min for furosemide and 9 min for the internal standard. The concentration of furosemide in samples was determined by comparing the furosemide/internal standard curves of peak height ratios versus furosemide concentration.

Results. To determine if rats were suitable to study the interaction between furosemide and phenytoin, rats were given phenytoin, 50 mg/kg, daily. At weekly intervals, the rats were given furosemide, 10 mg/kg, dissolved in the oral load of saline and renal activity of the diuretic determined. As shown in Table I, phenytoin did not significantly depress furosemide-induced diuresis after 1 week of pretreatment, whereas a significant decrease was seen after 2 or more weeks of pretreatment. Phenytoin treatment did not affect animal weights. After 3 weeks of pretreatment, the phenytoin group averaged 260 ± 11 g, whereas the nontreated group averaged 250 ± 8 g.

To assess more adequately the effect of phenytoin on the diuretic activity of furosemide when administered orally, rats pretreated with the anticonvulsant for 3–4 weeks were given an oral load of saline, 50 ml/kg, with various doses of the diuretic dissolved in the load. Renal excretion of the diuretic was also determined. These rats were compared to rats which received the phenytoin vehicle. As shown in the upper portion of Table II, phenytoin pretreatment resulted in a decrease in the diuresis induced by furosemide as expressed by urinary volume/3 hr, urinary volume as a percentage of the volume of the load, as well as $t_{1/2}$ (the time to excrete half of the volume of the load). Sodium excretion induced by furosemide was also decreased as indicated by the decrease in sodium excretion/3 hr and sodium excretion expressed as a percentage of that in the load of isotonic saline. The excretion of furosemide by the kidneys was also decreased. In addition to these effects, phenytoin also decreased urinary volume and the excretion of sodium in the group which received no furosemide.

TABLE I. EFFECT OF DURATION OF PRETREATMENT WITH PHENYTOIN, 50 mg/kg DAILY, ON THE DIURETIC ACTIVITY OF FUROSEMIDE, 10 mg/kg, ADMINISTERED ORALLY

Weeks of phenytoin pretreatment	Volume excreted in 3 hr – % oral load volume ± SEM	
	Vehicle[a]	Phenytoin[a]
1	57 ± 6.7	49 ± 6.7
2	70 ± 12.6	39 ± 2.3[b]
3	75 ± 9.0	34 ± 9.9[b]

[a] $N = 6$.
[b] Significantly different from phenytoin vehicle group.

Since this latter finding indicated that the antagonism by phenytoin of the actions of furosemide could be due to an effect on the absorption of furosemide as well as on the load of isotonic saline, a second series was performed in which both furosemide and the load of isotonic saline were administered intraperitoneally in order to bypass intestinal absorption. As indicated in the lower portion of Table II, phenytoin did not significantly alter the excretion of urinary volume or sodium in the group which received the furosemide vehicle. In the furosemide-treated groups, pretreatment with phenytoin did result in significant decreases in the excretion of urinary volume and sodium, although the magnitude of the decreases appeared to be less. The excretion of furosemide by the kidneys was not significantly altered.

Discussion. Pretreatment of rats with phenytoin significantly affects the diuretic activity of furosemide after a period of 2 weeks. Thus the rat can be used to study interactions of furosemide and phenytoin.

There appears to be more than one mechanism involved in the inhibition of the diuretic activity of furosemide by phenytoin. Since phenytoin decreased urinary volume and the excretion of sodium in rats not treated with furosemide when the load of isotonic saline was given orally, but not when the load was given intraperitoneally, there appears to be a general effect on absorption from the GI tract. Such an effect would decrease the activity of furosemide administered orally. In agreement with this is the decrease in the excretion of furosemide by the kidneys when the diuretic was given orally, but not when it was given intraperitoneally.

Phenytoin pretreatment also appears to directly interfere with the renal action of furo-

TABLE II. EFFECT OF PRETREATMENT WITH PHENYTOIN ON THE RENAL ACTIONS AND RENAL EXCRETION OF FUROSEMIDE ADMINISTERED ORALLY OR INTRAPERITONEALLY

		Urine volume			Excretion		
					Sodium		Furosemide
Treatment (mg/kg)	*N*	ml/3 hr	% of load	$t_{1/2}$[a] (min)	μeq/3 hr	% of load	(μg/3 hr)
Furosemide, oral							
0 + vehicle	4	7.4 ± 0.8[b]	48 ± 5.5	>180	584 ± 153	28 ± 7	—
0 + phenytoin	4	2.6 ± 0.4[c]	18 ± 3.9[c]	>180	133 ± 38[c]	7 ± 2[c]	—
12.5 + vehicle	5	12.7 ± 0.7	76 ± 4.0	76 ± 7.3	1167 ± 87	50 ± 4	178 ± 15
12.5 + phenytoin	5	2.9 ± 0.9[c]	20 ± 6.8[c]	>180[c]	268 ± 94[c]	13 ± 5[c]	77 ± 20[c]
25 + vehicle	5	15.5 ± 0.8	106 ± 9.6	45 ± 2.3	1530 ± 81	74 ± 6	374 ± 51
25 + phenytoin	5	6.8 ± 1.7[c]	50 ± 11[c]	142 ± 25[c]	501 ± 169[c]	26 ± 8[c]	132 ± 45[c]
50 + vehicle	5	20.7 ± 0.8	139 ± 6.9	40 ± 4.4	2132 ± 160	102 ± 7	618 ± 83
50 + phenytoin	5	11.8 ± 2.0[c]	85 ± 1.6[c]	92 ± 25	963 ± 189[c]	50 ± 10[c]	380 ± 74
Furosemide, ip							
+ vehicle	6	4.7 ± 0.4	28 ± 3.4	>180	121 ± 29	5 ± 1	—
0 + phenytoin	5	3.3 ± 0.4	22 ± 2.6	>180	76 ± 22	6 ± 2	—
12.5 + vehicle	6	22.4 ± 0.6	134 ± 4.9	41 ± 5.3	2287 ± 80	98 ± 5	1438 ± 115
12.5 + phenytoin	7	15.1 ± 1.5[c]	101 ± 13[c]	55 ± 7.8	1629 ± 152[c]	68 ± 4[c]	1255 ± 196
25 + vehicle	6	25.9 ± 1.3	157 ± 4.6	35 ± 2.2	2774 ± 130	119 ± 5	3494 ± 294
25 + phenytoin	6	19.1 ± 0.9[c]	123 ± 5.8[c]	42 ± 3.1	2010 ± 114[c]	94 ± 6[c]	2822 ± 823

[a] Time to excrete one-half of volume of load of isotonic saline.
[b] All values are means ± SEM.
[c] Significantly different from value above.

semide since a decrease in the renal activity of furosemide was seen when the diuretic was given intraperitoneally to phenytoin-pretreated rats. Burg *et al.* (4) have shown that the luminal concentration, rather than plasma or peritubular concentrations of furosemide, is related to the diuretic's inhibitory action on sodium chloride reabsorption. Since furosemide is highly bound to plasma proteins, very little of the drug enters the tubules by filtration. The diuretic enters the tubules primarily by active secretion by the organic anion secretory mechanism in the proximal tubules (5). An interference by phenytoin of the secretion of furosemide does not appear likely since the renal excretion of furosemide (and hence luminal access of furosemide) was not significantly altered. Thus it would appear that phenytoin interacts with furosemide at the site where the diuretic affects. sodium chloride reabsorption.

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