Kaliuretic Properties of Diuretic Agents Measured by Isotope Dilution

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Abstract ☐ The isotope dilution technique was utilized to determine the effect of chronic furosemide and hydrochlorothiazide administration on total exchangeable potassium in the rat. The results obtained in the investigation indicated that the chronic administration of the diuretic agents did not significantly alter exchangeable potassium in the animals.

Keyphrases ☐ Diuretic agents—kaliuretic properties ☐ Potassium, exchangeable—diuretic effect ☐ Isotope dilution—analysis method ☐ Liquid scintillation counting—radioactivity determination ☐ Flame photometry—analysis

In the search for new and improved diuretics, the excretion and depletion of body electrolytes by these therapeutic agents must be evaluated. The effect of diuretics on electrolyte balance in dogs has been determined by the Van Arman method or modifications thereof (1–4), while in rats the Lipschitz method, or modifications of it, has been employed (2, 5–9). Recently, radiotracer techniques utilizing *in vivo* whole-body liquid scintillation counting have been used in studying the effects of diuretics on electrolyte balance. Using ^{22}Na, Rupe *et al.* (10) evaluated compounds for their natriuretic and diuretic activity in rats. Born *et al.* (11) using ^{42}K, studied the kaliuretic properties of furosemide and hydrochlorothiazide in the rat. Shaw *et al.* (12) investigated the kaliuretic and natriuretic properties of furosemide with ^{42}K and ^{24}Na in separate studies in swine. Born *et al.* (13) developed an *in vivo* whole-body liquid scintillation counting technique for the simultaneous determination of the kaliuretic and natriuretic properties of furosemide and hydrochlorothiazide by using ^{42}K and ^{24}Na and dual-channel radionuclide counting. The whole-body liquid scintillation counting technique allowed the determination of the effect of the acute administration of furosemide and hydrochlorothiazide on electrolyte balance. However, since many disease states necessitate chronic diuretic therapy, the determination of the long-term effect of diuretic therapy on electrolyte balance, especially that of potassium, is also of utmost importance. The determination of exchangeable potassium in an animal allows the measurement of whole-body potassium content before and during diuretic therapy. Exchangeable potassium can be measured by the isotope-dilution technique (14–17). In the isotope-dilution technique, a known quantity of radionuclide is administered to an animal. The radionuclide is allowed to exchange with the naturally occurring element, during which time the excretion of the radionuclide is measured. When an equilibrium is obtained, as indicated by a constant ^{42}K specific activity of successive urine collections, the quantity of the element in which the isotope is diluted is inversely proportional to the concentration of the radionuclide in the stable element (specific activity) of any body sample. The quantity of stable element is referred to as the body content of exchangeable element which, in the case of complete exchange, is the total amount of the element in the body. The purpose of the following research was to utilize the isotope-dilution technique to determine the effect of chronic furosemide or hydrochlorothiazide administration on total-body exchangeable potassium in the rat.

EXPERIMENTAL PROCEDURES

A total of 30 female Sprague-Dawley strain[1] albino rats was divided into five groups of six animals. Animals were housed individually in stainless steel screenwire-bottom metabolism cages. Distilled water and food were allowed *ad libitum* throughout the experimental period, except for 2 days of fasting during each exchangeable potassium determination. For an exchangeable potassium determination, all animals were administered 250 μc. of ^{42}K by intraperitoneal injection. A 24-hr. period was allowed for equilibration of ^{42}K with naturally occurring potassium. The rate of exchange of ^{42}K with naturally occurring potassium in the animal body is high. Approximately 24 hr. after the administration of ^{42}K a complete exchange is attained for all tissues except the brain and erythrocytes (14, 18). Following the equilibration period, urine was collected for 24 hr. at 8- or 12-hr. intervals. Each urine sample was measured by volume and ^{42}K content determined by external sample liquid scintillation counting (11). Total potassium in each urine sample was determined flamephotometrically[2] using the potassium emission-line spectrum of 768 mμ. A calibration curve was prepared with potassium standard solutions containing KCl and NaCl at a molar ratio of 1 to 4.

Exchangeable potassium was calculated as follows:

$$Ke = \frac{{}^{42}K_i - {}^{42}K_0}{{}^{42}K_u/{}^{39}K_u}$$

where Ke = meq. of exchangeable potassium in the animal; $^{42}K_i$ = potassium-42 (c.p.m.) administered to the animal; $^{42}K_0$ = potassium-42 (c.p.m.) excreted in the urine to the time of equilibrium urine collection; $^{42}K_u$ = potassium-42 (c.p.m.)/l. in the urine sample; $^{39}K_u$ = total potassium (meq./l.) in the urine sample; $^{42}K_u/^{39}K_u$ = specific activity of the equilibrium urine sample (c.p.m./meq.).

For each group of animals, exchangeable potassium levels were determined before initiating control or diuretic treatment (time zero). After the determination of exchangeable potassium at time zero, Group 1 animals were given a daily oral dose of furosemide[3] at a level of 50 mg./kg./day, while Group 2 rats were treated with hydrochlorothiazide[4] at the same dose level. Exchangeable potassium was again determined 14 days and 28 days after time zero. Following the determination of exchangeable potassium at time

[1] Sprague-Dawley, Inc., Madison, Wisc.

[2] Beckman DU spectrophotometer model 2400, Beckman Instruments, Inc., Fullerton, Calif.

[3] Furosemide is 4-chloro-*N*-furfuryl-5-sulfamoylanthranilic acid, and is marketed as Lasix by Hoechst Pharm. Co., Cincinnati, Ohio.

[4] Hydrochlorothiazide is 6-chloro-3, 4-dihydro-7-sulfamoyl-2*H*-1,2,4-benzothiadiazine 1,1-dioxide. The compound was supplied by Merck, Sharpe & Dohme, West Point, Pa., as Hydrodiuril.

Table I—Effect of Chronic Diuretic Treatment on Exchangeable Potassium

Drug Treatment, Days	Total Exchangeable Potassium, meq.[a] Furosemide	Hydrochlorothiazide	Control
	Group 1	**Group 2**	
0	10.04 ± 0.58	10.39 ± 1.00	—
14	12.98 ± 0.71	13.09 ± 1.22	—
28	15.41 ± 1.38	13.69 ± 1.81	—
	Group 3	**Group 4**	**Group 5**
0	16.09 ± 1.24	15.02 ± 0.53	15.09 ± 0.78
12	14.78 ± 0.79	14.67 ± 0.84	15.67 ± 0.71
26	14.62 ± 0.59	14.55 ± 0.38	14.33 ± 0.41

[a] Average of six animals per group ± standard deviation.

zero, Group 3 animals were given a daily oral dose of furosemide at a level of 50 mg./kg./day, while Group 4 rats were treated with hydrochlorothiazide at the same dose level. Group 5 animals served as controls. Exchangeable potassium was again determined 12 days and 26 days following initiation of drug or control treatment. The diuretic dose level was selected because of the definite, almost equivalent, natriuretic activity of furosemide and hydrochlorothiazide in the rat at 50 mg./kg. (19).

RESULTS AND DISCUSSION

As indicated previously, urine was collected after the equilibrium period for 24 hr. at 8- or 12-hr. intervals and exchangeable potassium was determined for a particular animal with each urine sample. Although the data are not presented, the exchangeable potassium values determined from the two or three urine samples obtained from a single animal at a particular interval of study showed highly satisfactory agreement, thus, indicating a continuity of assay as well as the establishment of a potassium equilibrium. Data are expressed as the average total exchangeable potassium (meq.) per group of animals in Table I. Exchangeable potassium levels in furosemide (Group 1)- and hydrochlorothiazide (Group 2)-treated animals were not significantly different (Student *t* test) at any interval of determination. At each interval of study, a comparison of the exchangeable potassium level in the furosemide-treated animals (Group 3) to the exchangeable potassium level in the hydrochlorothiazide-treated rats (Group 4) revealed no significant difference between the groups. Also, the exchangeable potassium levels in Groups 3 and 4 were not significantly different than observed in the control animals (Group 5).

Total exchangeable potassium levels determined at time zero for animals in Groups 1 and 2 varied considerably from values obtained for animals in Groups 3–5. The variation can be attributed to the difference in the initial weight of animals in Groups 1 and 2 (176–180 g.) as compared to animals in groups 3–5 (225–288 g.). The increase in total exchangeable potassium levels in Groups 1 and 2 animals during the interval of drug treatment can be related to the increasing weight of the rats. The body weight of animals in Groups 3, 4, and 5 remained relatively constant throughout the study. In order to eliminate weight variation, the data were expressed as exchangeable potassium per kilogram of body weight. Exchangeable potassium levels (meq./kg.) in furosemide (Group 1)- and hydrochlorothiazide (Group 2)-treated animals were not significantly different at any interval of determination. At each interval of study, no significant differences in exchangeable potassium levels (meq./kg.) were observed between Groups 3, 4, and 5.

The results obtained in the investigation indicated that the chronic administration of either furosemide or hydrochlorothiazide did not significantly alter exchangeable potassium in the rat. Since earlier studies have shown an increased excretion of potassium from the acute administration of furosemide and hydrochlorothiazide (11, 13), the results of this investigation illustrate the importance of the isotope-dilution technique for the determination of the long-term effect of diuretic treatment on electrolyte balance.

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