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Role of Carbonic Anhydrase in Bone: Partial Inhibition of Disuse Atrophy of Bone by Parenteral Acetazolamide

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Summary. Carbonic anhydrase inhibitor acetazolamide blocks the hypercalcemic response to parathyroid hormone (PTH) and to dibutyryl 3′,5′-cyclic AMP in the nephrectomized-parathyroidectomized rat. In addition, we have reported that acetazolamide, when incorporated in the diet, partially prevents denervation-induced bone loss in a rat model of disuse osteoporosis. The present study compares the effectiveness of orally and subcutaneously administered acetazolamide in preventing denervation-induced bone loss in the rat model. The rats were treated with acetazolamide either orally, by incorporation in the diet of concentrations of 0.2, 0.5, or 1.5% for 15 days, or parenterally by two different subcutaneous methods of administration. The latter included either injection twice daily for 15 days or continuous infusion for 8 days using an osmotic minipump. It was found that parenteral administration was as effective in partially preventing denervation-induced bone mass changes as oral administration. In addition, protection by the parenteral route could be accomplished with much smaller daily doses; continuous infusion required the least daily dose. Approximately 50% protection was observed to occur with daily doses of 1,094, 129, and 8 mg/kg body weight for the oral, subcutaneous injection, and subcutaneous infusion routes respectively. These findings are consistent with our concept that carbonic anhydrase plays a significant role in bone metabolism.

Key words: Acetazolamide — Denervation-induced bone loss — Oral ingestion — Subcutaneous injection — Continuous subcutaneous infusion.

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Earlier reports from our laboratory have indicated that the carbonic anhydrase inhibitor acetazolamide has important effects on bone and calcium metabolism. Acetazolamide inhibits the hypercalcemic response to parathyroid hormone (PTH) and also to dibutyryl 3′,5′-cyclic AMP in the nephrectomized–parathyroidectomized rat [1, 2] and, when incorporated in the diet, partially blocks the development of disuse atrophy of bone in a rat model [3]. These observations led to our suggestion that skeletal carbonic anhydrase, reported to be present in osteoclasts [4], might play a significant role in the mechanism of bone resorption [1].

Other investigators have made relevant contributions in this area. Acetazolamide, when added to bone cultures, *in vitro*, inhibits PTH-induced bone resorption [5–7], an effect which has been recently confirmed in our laboratory [8]. Waite [9] has reported that inhibition of PTH-induced hypercalcemia in nephrectomized-parathyroidectomized rats is also seen with other carbonic anhydrase inhibitors (methazolamide and ethoxolamide). The relative potencies of this antagonism paralleled the potencies of carbonic anhydrase inhibition; the inactive butyl analogue of acetazolamide, CL 13,850 (N-t-butyl-acetazolamide), failed to inhibit the PTH-induced hypercalcemia [9]. Lineberry and Waite [10] have additionally shown that, if the renal-mediated metabolic acidosis induced by acetazolamide is neutralized by administration of a buffer, acetazolamide causes a marked hypocalcemic response even in *intact* rats.

More recently, attention has turned to the effects of acetazolamide on calcium mobilization from bone induced both *in vivo* and *in vitro* by calcitriol (1,25-dihydroxyvitamin D_3). Carbonic anhydrase inhibitors antagonized the hypercalcemic response to calcitriol *in vivo* [11]. This finding is in concert with the *in vitro* observation of Hall and Kenny [12]

that calcitriol-induced calcium release from neonatal mouse calvarial cultures is completely inhibited by the addition of 10^{-4} M acetazolamide to the medium.

The present study was undertaken to compare the effectiveness of acetazolamide in preventing denervation-induced bone loss when the drug is administered either by the oral or by the parenteral route. A preliminary report of this work has appeared elsewhere [13].

Materials and Methods

Male rats of the Sprague-Dawley strain were obtained from the Charles River Breeding Laboratories (Wilmington, MA) and were maintained on a commercial chow (Purina Rodent Chow, Ralston Purina, St. Loius, MO, or Wayne Lab-Blox, Allied Mills, Chicago, IL) in suspended stainless steel wire-mesh cages in an environment with a 12-h light/12-h dark cycle (0700 h on, 1900 h off). Purina Rodent Chow was used unless indicated otherwise. The rats were randomized into groups on the basis of body weight.

Denervation of the forelimb and preparation of the bone sample were performed as described by Conaway et al. [3]. The difference between the defatted dry weight of the bones removed from the intact and denervated forelimbs was used as the index of the denervation response.

In all experiments involving administration of acetazolamide by both oral and continuous subcutaneous infusion, the rats were pair-fed in the usual fashion [14]; those rats receiving acetazolamide, or the higher dose of acetazolamide, were used as the pace-setters. Acetazolamide sodium was obtained from Lederle Laboratories (Pearl River, NY) through the courtesy of Dr. Ira Ringler.

Dietary Acetazolamide

All rats upon delivery were fed regular laboratory chow for 1 week after which they were randomized into the appropriate control and experimental groups. At this point all rats were placed on ground laboratory chow, and pair-feeding was begun in order to acclimate the rats to ground chow and to obtain baseline food consumption data. One week after initiating pair-feeding, the rats were weighed (day 0), subjected to forelimb denervation, and placed on the experimental diets consisting of ground laboratory chow containing either zero, 0.2, 0.5, or 1.5% acetazolamide sodium for a period of 15 days. At the end of this period the rats were weighed (day 15) and bled by cardiac puncture under light ether anesthesia for determination of plasma calcium. Both humeri were removed following ether euthanasia for measurement of the bone mass difference between the intact and denervated limbs. A total of four experiments (0-116, 0-208, 0-226, R-2) were conducted.

Subcutaneous Injection

Continuous subcutaneous infusion was performed by means of an Alzet® osmotic minipump (Model 1701, Alza Corp., Palo Alto, CA) containing the acetazolamide solution. The minipump had a capacity of 170 μl and was purported to pump at a rate of approximately 1 μl/h for a total duration of around 7 days. The minipump was implanted subcutaneously in the dorsal neck region. In all subcutaneous experiments (injection and continuous infusion) acetazolamide was dissolved in dilute NaOH adjusted to pH 9.0. Control solutions were 0.154 M NaCl. Distilled water was used for drinking in all experiments.

All rats upon delivery were fed regular laboratory chow for 4 days after which they were randomized into the appropriate control and experimental groups. After an additional 7 days on regular chow the rats were weighed (day 0), subjected to forelimb denervation, and given subcutaneous injections of acetazolamide sodium twice daily (0900 and 1400 h). Each of the two daily doses consisted of 0.5 ml/rat of a solution containing either 20 or 100 mg/ml of the drug. Control rats received injections of 0.154 M NaCl. During the 15-day injection period, the rats were deprived of food between 0800 and 1700 h daily. The last injections were given on day 14 and on day 15 the rats were weighed and bled by cardiac puncture under light ether anesthesia for determination of plasma calcium. Both humeri were removed for measurement of the bone mass difference. At no time were the rats pair-fed.

Continuous Subcutaneous Infusion

All rats upon delivery were placed on commercial chow (Wayne Lab-Blox in experiments R-30 and R-44) for 5 days (except experiment R-44 which was 1 day) after which they were randomized into the appropriate control and experimental groups. At this point all rats were placed on ground commercial chow for a period ranging from 3 to 6 days at which time (day −1) the rats were randomized into appropriate control and experimental groups and pair-feeding was begun in the usual fashion [14]. The next day (day 0) the rats were weighed, subjected to forelimb denervation, and implanted subcutaneously with an osmotic minipump containing either 5, 50, 500, or 1,000 mg/ml of acetazolamide sodium. Control rats received no treatment. Eight days (day 8) following denervation and implantation of the minipump, the rats were weighed and bled by cardiac puncture under light ether anesthesia for determination of plasma calcium and inorganic phosphate concentrations. Both humeri were removed for measurement of the bone mass difference.

Plasma Calcium and Inorganic Phosphate

Plasma calcium was determined by atomic absorption spectrophotometry (Model 303, Perkin-Elmer, Norwalk, CT) following dilution in 0.5% lanthanum oxide dissolved in dilute hydrochloric acid. Plasma inorganic phosphate was determined using a Technicon AutoAnalyzer II (Technicon Instruments, Tarrytown, NY).

Statistical Analyses

Statistical analyses were performed using Student's t test [15].

Results

Body weight, food consumption, and plasma calcium data for two of the experiments (0-226, R-2) are presented in Table 1. In both experiments, none

Table 1. Body weights, food consumption, and plasma calcium concentrations in male rats treated with *dietary* acetazolamide for 15 days

Expt. no.	Treatment	Body weight		Food consumption		Plasma Ca
		Day 0	Day 15	Day 0	Day 15	Day 15
		(g)		(g/day)		(mg/dl)
0-226	Control	207 ± 4.4	233 ± 10.1	22 ± 0.8	14 ± 1.2[b]	9.3 ± 0.10
	0.2% acetazolamide	205 ± 3.6	223 ± 8.5	22 ± 0.8	14 ± 1.0[b]	9.6 ± 0.20
	0.5% acetazolamide	212 ± 5.9	227 ± 11.3	22 ± 0.9	14 ± 1.6[b]	9.5 ± 0.15
R-2	Control	182 ± 3.0	181 ± 6.9	20 ± 0.8	12 ± 0.6[b]	9.6 ± 0.11
	0.5% acetazolamide	185 ± 3.8	173 ± 5.5	19 ± 0.9	12 ± 0.5[b]	10.0 ± 0.18
	1.5% acetazolamide	188 ± 4.3	175 ± 5.7	16 ± 1.3	12 ± 0.6[a]	9.9 ± 0.12

Rats were "pair"-fed using higher acetazolamide dose as the pace-setter
Each group contained 10 rats; all values are means ± SE
[a] $P < 0.05$, [b] $P < 0.001$ when compared with day 0

of the acetazolamide treatments resulted in any significant differences in body weights at day 15 from the respective control groups, indicating the effectiveness of the pair-feeding technique (Table 1). Within each group the body weights at day 0 and day 15 did not differ significantly, indicating no significant gain or loss in weight in any of the groups. All groups in both experiments exhibited a significant decline in food consumption at day 15 of treatment when compared with day 0, the time when the acetazolamide treatments were begun (Table 1). Acetazolamide treatment had no effect on plasma calcium concentrations regardless of the dose. None of these data is in conflict with our previous publication in which we reported the effects of 0.05, 0.2, and 0.5% acetazolamide on various parameters including body weight, food consumption, and plasma calcium [16].

The bone response data were combined from four experiments and are presented in Fig. 1. Dietary acetazolamide reduced the bone mass loss in a dose-dependent fashion. Whereas incorporation of the drug in the diet at 0.2% had no significant effect, concentrations of 0.5% and 1.5% resulted in significant reductions of bone mass loss of 17 and 47% respectively (Fig. 1).

Body weight and plasma calcium data are presented in Table 2. Acetazolamide treatment had no effect on the plasma calcium concentration. On day 15, body weights were significantly lower in the rats treated with the higher dose of acetazolamide reflecting the absence of pair-feeding. The bone response data are presented in Fig. 2. Both doses of acetazolamide significantly reduced the bone mass loss; the 20 and 100 mg/day doses resulted in significant reductions of 31% and 45% respcetively (Fig. 2).

Body weight and food consumption data for the three experiments—rats treated with dietary acetazolamide by the oral route, by subcutaneous injections, and by continuous subcutaneous infusion—(R-58, R-44, R-30) are presented in Table 3; plasma calcium and inorganic phosphate data are presented in Table 4. There were no significant differences on day 0 between the mean values for body weight or food consumption data in any of the groups within each of the three experiments (Table 3). Similarly, no significant differences were found at day 8 indicating the effectiveness of the pair-feeding procedure. None of the doses of acetazolamide had any significant effect on the plasma calcium concentration; the plasma inorganic phosphate level was significantly increased only by the highest dose of acetazolamide (Table 4).

The bone response data were combined from the three experiments and are presented in Fig. 3. Whereas the lowest dose (5 μg/h) of acetazolamide sodium exhibited no protection, the three highest doses (50, 500, and 1,000 μg/h) reduced the bone mass loss resulting in significant reductions of 40%, 56%, and 50% respectively. The 50 μg/h dose in one experiment (R-44) resulted in 49% protection. Based on the final mean body weight (146 g) in this group, this dose represents 8.2 mg kg^{-1} day^{-1} of acetazolamide sodium.

Comparison of Administration Routes

A comparison of the doses required to effect significant reductions in bone mass loss by the three types of administration is presented in Fig. 4. Protection by the parenteral route was accomplished with smaller daily doses than by the oral route. Continuous infusion required the least daily dose. Approximately 50% protection was observed to

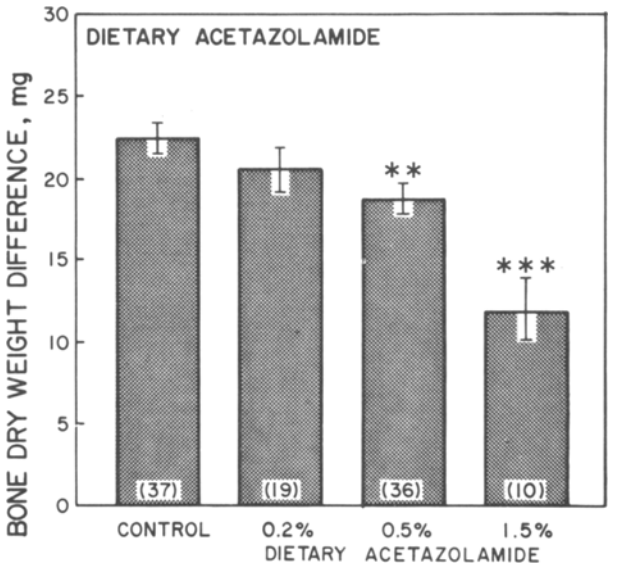

Fig. 1. Effects of acetazolamide sodium incorporated in ground diet at zero (*control*), 0.2%, 0.5%, and 1.5% concentrations on the bone dry weight difference between humeri removed from the intact and denervated forelimbs of each rat after 15 days on the respective diets. The data were combined from four experiments; pair-feeding was used in each experiment. The number of rats in each combined group is in parentheses; the variations about the means are standard errors. Significant differences from the control groups are indicated (**$P < 0.01$; ***$P < 0.001$).

occur with daily doses of 1,029, 129, and 8 mg/kg body weight for the oral, subcutaneous injection, and continuous subcutaneous infusion routes respectively.

Discussion

The present work clearly documents that the carbonic anhydrase inhibitor, acetazolamide, when administered to rats by the parenteral route, is able to prevent denervation-induced bone mass loss in a rat model of disuse atrophy of bone. More importantly, the protection afforded is able to be accomplished with daily doses that are over 100 times less than the doses required by the oral route. Two parenteral routes were examined. The first route, subcutaneous injections given twice daily during a 9-hour period of food deprivation, resulted in approximately 50% protection when administered at a total daily dose of 129 mg/kg for 15 days. This dose was more than 8-fold less than that required by the oral route (1,094 mg/kg).

The rationale behind the design of the subcutaneous injection experiment was as follows. The oral route, accomplished by incorporation of the acetazolamide in the diet, would present the rat with the drug only during times of feeding. These times obviously coincide with periods of calcium intake rather than with periods of calcium deprivation. It was reasoned that the latter periods might be ones, when bones, subjected to less use and therefore more susceptible to bone loss, might be in greater need of the protective effects of the drug, which is known to have a relatively short plasma half-life. Although rats are generally considered to be nocturnal feeders, this natural pattern was reinforced in the experimental design by deprivation of food during the daylight hours, the period in which the injections were made.

Further work along these lines was discontinued when it was discovered that parenteral administration of acetazolamide by continuous subcutaneous infusion afforded similar protection from bone loss at exquisitely low daily doses of only 8 mg/kg, a dose reduction that represents a greater than 125-fold improvement when compared with dietary administration. It may be anticipated from this important finding that such low doses, and therefore presumably low plasma acetazolamide concentrations, will exhibit minimal, if any, of the pathophysiological changes associated with toxic doses of acetazolamide, such as renal calcification and metabolic acidosis. Our preliminary report [20] supports this contention and future detailed reports will document this further.

When these *in vivo* findings are discussed in the context of our *in vitro* work, they assume even greater importance. Using the neonatal mouse calvarial *in vitro* model, we have recently presented preliminary reports that several calcemic agents, such as parathyroid hormone, calcitriol, dibutyryl cyclic AMP, and forskolin, all of which increase calcium mobilization from bone into medium, also increase the skeletal content of carbonic anhydrase activity [8, 12, 17, 18]. In addition, the calcium-mobilizing effect of these agents is blocked completely by the addition of appropriate concentrations (10^{-4} M) of the carbonic anhydrase inhibitor, acetazolamide. The inactive analogue of acetazolamide, CL 13,850 (N-t-butylacetazolamide) at similar concentrations, fails to prevent calcemic agent-induced bone resorption.

One important question needs to be addressed. In the *in vivo* rat model of disuse osteopenia, acetazolamide, whether it was given orally (Fig. 1), parenterally by subcutaneous injection (Fig. 2), or by continuous subcutaneous infusion (Fig. 3), seldom afforded more than 50% protection. Acetazolamide on the other hand, when added to the *in vitro* neonatal mouse calvarial model, has consistently exhibited 100% inhibition of agent-induced bone resorption. Why does acetazolamide, then, fail to inhibit completely the bone loss in the *in vivo*

Table 2. Body weights and plasma calcium concentrations in male rats treated with acetazolamide by *subcutaneous injections* twice daily for 15 days

Expt. no.	Treatment	Dose SC (mg/day)	No. rats	Body weight Day 0 (g)	Body weight Day 15 (g)	Plasma Ca (mg/dl)
R-18	Control	—	5	145 ± 10.5	223 ± 16.9	10.0 ± 0.21
	Acetazolamide	20	6	140 ± 6.6	210 ± 10.1	10.2 ± 0.10
	Acetazolamide	100	5	144 ± 8.6	155 ± 13.3[a]	9.5 ± 0.17

Values are means ± SE
Rats injected subcutaneously (0.5 ml/rat) twice daily (0900 and 1400 h)
Rats were deprived of food between 0800 and 1700 h daily
[a] $P < 0.05$ when compared with day 15 control group

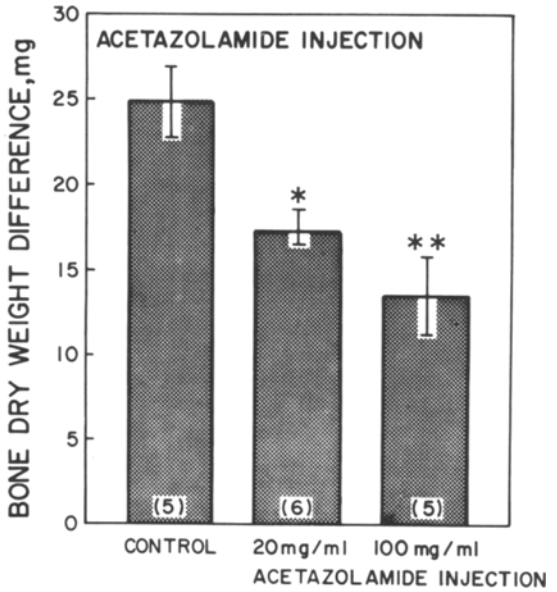

Fig. 2. Effect of acetazolamide sodium administered twice daily (0900 h and 1400 h) by subcutaneous injection on the bone dry weight difference after 15 days of treatment. The number of rats in each group is in parentheses; the variations about the means are standard errors. Significant differences from the control group are indicated (*$P < 0.05$; **$P < 0.01$).

model? Several explanations may be advanced. First, the concentration of free (unbound) acetazolamide bathing the bone tissue may be insufficient to effect adequate inhibition of the enzyme *in vivo*. Secondly, even if complete inhibition is effected, the uncatalyzed reaction is notoriously rapid and may be fast enough to supply carbonic acid at a rate sufficient for *in vivo* needs. Lastly, the denervation-induced bone loss in the *in vivo* rat model most likely has complex origins involving not only effects mediated by bone cells and arising from disuse *per se*, but also from the repair or RAP phenomenon of Frost [19] in response to the injury of denervation, from hemodynamic or from other phenomena. Let us briefly examine each of these possibilities.

How do the *in vivo* circulating levels of acetazolamide compare with the 10^{-4} M concentration used *in vitro*? In a preliminary report [20] we have claimed that plasma acetazolamide levels of around 225 ng/ml are effective *in vivo* in reducing denervation-induced bone loss. Such concentrations translate to around 1×10^{-6} M; free or unbound concentrations of acetazolamide would be even less due to binding to plasma proteins. This greater than 100-fold discrepancy between *in vivo* and *in vitro* drug concentrations, may therefore, be of some significance in the difference in bone loss protection exhibited by the two situations. Militating against the possibility that this discrepancy is of major significance is the fact that very high doses of acetazolamide (such as 1,000 μg/h compared with the 50 μg/h that generated the 225 ng/ml plasma concentrations) afforded no greater protection (Fig. 3).

It is unlikely that the rapidity of the uncatalyzed reaction explains the difference in the degree of inhibition exhibited by acetazolamide in the two models as this should play a similar role in both situations. Nevertheless, the concentrations of the important reactant or substrate, namely carbon dioxide, may differ significantly in the two situations. The concentration of CO_2 is known with precision (5%) *in vitro* but is essentially unknown at the appropriate bone site *in vivo*. The concentration of CO_2 does influence the *in vitro* response to calcemic agents and also the concentrations of acetazolamide required to inhibit this response [6].

The most likely explanation for the discrepancy

Table 3. Body weights and food consumption in male rats treated with acetazolamide by *continuous subcutaneous infusion* (minipump) for 8 days

Expt. no.	Treatment	Minipump dose (μg/h)	Body weight (g)		Food consumption (g/day)	
			Day 0	Day 8	Day 0	Day 8[a]
R-58	Control	—	170 ± 3.0	209 ± 3.9	18 ± 1.5	19 ± 0.8
	Acetazolamide	5	170 ± 2.5	197 ± 8.5	15 ± 1.7	19 ± 0.9
	Acetazolamide	50	168 ± 2.2	213 ± 7.1	16 ± 1.0	18 ± 0.4
R-44	Control	—	134 ± 4.0	148 ± 5.1	12 ± 1.4	16 ± 0.7
	Acetazolamide	50	138 ± 5.0	146 ± 5.1	14 ± 0.9	17 ± 0.4
	Acetazolamide	500	136 ± 3.4	156 ± 5.0	14 ± 0.8	16 ± 0.4
R-30	Control	—	193 ± 5.6	222 ± 6.5	14 ± 1.5	19 ± 0.4
	Acetazolamide	1,000	202 ± 3.5	238 ± 5.4	16 ± 1.0	19 ± 0.4

Rats were "pair"-fed using higher acetazolamide dose as pace-setter
Values are means ± SE; 7 rats/group, except experiment R-30 (5 rats/group)
Minipumps: 170 μl capacity; 1 μl/h rate
[a] Day 7 in experiments R-58 and R-44

Table 4. Plasma calcium and inorganic phosphate concentration in male rats treated with acetazolamide by *continuous subcutaneous infusion* (minipump) for 8 days

Expt. nos.	Treatment	Minipump dose (μg/h)	Plasma Ca (mg/dl)	Plasma P (mg/dl)
R-30, R-44, R-58	Control	—	9.5 ± 0.14 (18)	9.0 ± 0.44 (17)
R-58	Acetazolamide	5	9.5 ± 0.23 (7)	9.4 ± 0.32 (7)
R-44, R-58	Acetazolamide	50	9.3 ± 0.14 (14)	9.2 ± 0.36 (14)
R-44	Acetazolamide	500	9.2 ± 0.15 (7)	8.2 ± 0.30 (6)
R-30	Acetazolamide	1,000	9.9 ± 0.28 (4)	10.4 ± 0.40 (3)[a]

Rats were "pair"-fed in the respective experiments using the rats treated with acetazolamide (higher dose in R-58 and R-44) as the pace-setters
Values are means ± SE; no. of rats in parentheses
Minipumps: 170 μl capacity; 1 μl/h rate
[a] $P < 0.05$ when compared with control group

is the complexity of the *in vivo* model when compared with the greater simplicity of the *in vitro* situation. Hemodynamic phenomena alone, resulting either directly from denervation or indirectly from the associated trauma, may account in large measure for the discrepancy. For example, previous work from our laboratory has shown that the catecholamines, such as epinephrine and isoproterenol, have significant effects on calcium and bone metabolism [2, 21–33]. More specifically, epinephrine exerts a significant hypercalcemic response in both parathyroidectomized rats [22] and parathyroidectomized-nephrectomized rats [2]; the latter response is not blocked by doses of acetazolamide which inhibit the hypercalcemic responses to parathyroid hormone [2], dibutyryl cyclic 3′,5′-adenosine monophosphate [2], and to calcitriol [11]. These findings suggest that epinephrine mediates its *in vivo* hypercalcemic response through mechanisms that do not involve carbonic anhydrase. Effects on bone blood flow are a more likely basis for the hypercalcemic response to catecholamines. The order of potency with respect to the latter response (isoproterenol > epinephrine > norepinephrine) [22] lends support to the concept and adds credence to the possibility that alterations in bone blood flow resulting from limb denervation may contribute significantly to the mechanisms underlying the bone loss. If so, it cannot be expected that inhibition of carbonic anhydrase by acetazolamide would result in complete protection in the *in vivo* model.

What relationships, if any, do these findings have

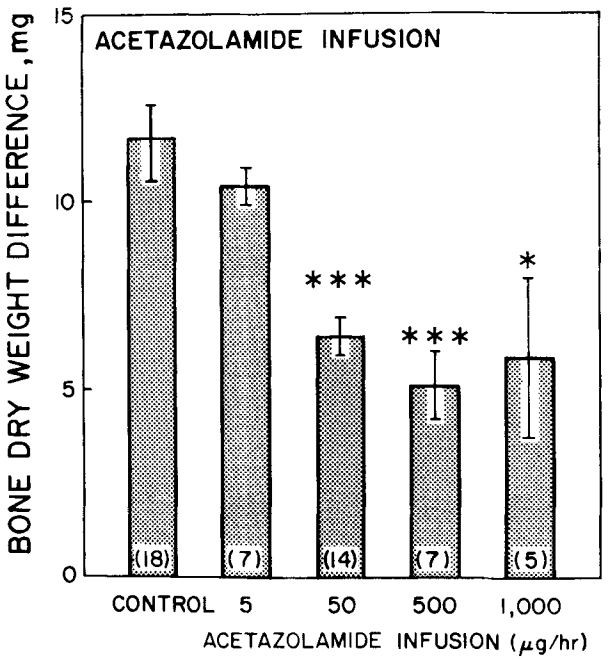

Fig. 3. Effects of acetazolamide sodium administered by continuous subcutaneous infusion using a subcutaneously implanted osmotic minipump with a rate of 1 μl/h on the bone dry weight difference after 8 days of treatment. The data were combined from three experiments; pair-feeding was used in each experiment. The number of rats in each group is in parentheses; the variations about the means are standard errors. Significant differences from the control groups are indicated (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

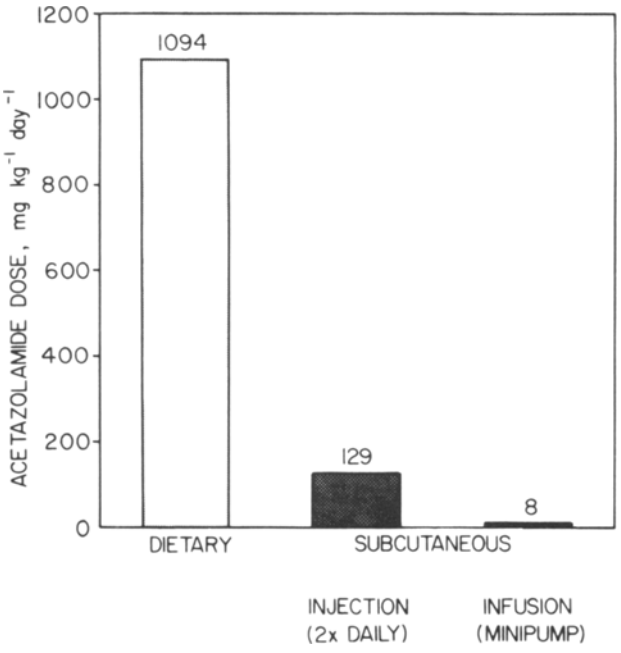

Fig. 4. Comparison of daily doses of acetazolamide sodium required to give approximately 50% protection in the rat model of disuse atrophy of bone by three different routes: oral (*dietary*) administration by incorporation in the diet (left-hand bar); *subcutaneous* injection twice daily (middle bar); and continuous subcutaneous *infusion* by use of an osmotic minipump (right-hand bar).

to human disorders of bone and calcium metabolism? The rat model used in this study is far from ideal with respect to extrapolation to human bone; such extrapolations, when made, should be done cautiously and soberly. Nevertheless, the recent clincial report that a genetic deficiency in a carbonic anhydrase isoenzyme, carbonic anhydrase II, is associated with osteopetrosis [24] suggests that skeletal carbonic anhydrase may have clinical relevance. If true, then acetazolamide may prove useful therapeutically in combating bone loss in a variety of clinical situations whether these be associated with prolonged bedrest, drug-induced osteoporosis, or hormonal changes seen in postmenopausal osteoporosis. Although interspecies comparisons may be hazardous, it is worth noting that the plasma concentrations of acetazolamide associated with bone loss protection in the rat, namely around 225 ng/ml [20], are more than 40-fold less than plasma concentrations normally encountered in the human (1.0–1.5 mg/dl or 10,000–15,000 ng/ml) when acetazolamide is being used as a therapeutic agent. It is possible that plasma levels of acetazolamide required for reduction of bone loss in the human might prove to be several fold less than the 1.0–1.5 mg/dl needed for other therapeutic purposes. If this were the case, reduction of bone loss might be achieved with even less toxicity than is seen with current clinical doses of acetazolamide.

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