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Differential Response of Muscle and Gastric Histidine Decarboxylase to Compound 48/80 and Dietary Calcium[1] (42171)

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Abstract. Previous work from this laboratory had indicated that *in vivo*, histidine decarboxylase (HDC) activity was stimulated by compound 48/80 in rat leg muscle, and that high dietary calcium had a stimulating effect on gastric HDC activity. In the present investigations the 48/80 effect was also observed *in vitro* in leg muscle extracts from rats, chicks, and guinea pigs. Compound 48/80 had no effect *in vitro* on histamine metabolism of gastric tissue homogenates in any of the animal species studied. A dietary effect of high calcium intake was noted in rat gastric tissue but not in rat leg muscle. *In vitro* addition of 48/80 and/or calcium had no stimulatory effect on bacterial HDC or on muscle carnosinase activity. These findings, in conjunction with a comparison of stomach and leg muscle mast cell populations, confirm an HDC stimulatory role for 48/80 in muscle, in addition to its histamine-releasing function from mast cells.

The importance of histamine (HM) metabolism in brain, stomach, skin, and mast cells has been well documented (1–5). There is an abundance of evidence suggesting that in these tissues compound 48/80 exerts an indirect, releasing effect, on HM metabolism. In contrast, very little work has been reported on muscle HM metabolism in general and on the role of compound 48/80 in particular. Muscle has been shown to be an important source of HM following trauma, and HM synthesis in muscle appears to regulate the rate of tissue repair (6). Of the few studies with muscle tissue, work from this laboratory (6–8) has pointed toward a direct, histidine decarboxylase (HDC) stimulatory role for compound 48/80 *in vivo*.

The objectives of the present study were to investigate and enlarge our understanding of (a) stomach and muscle HM metabolism in rat, chicken, mouse, and guinea pig, with particular reference to the role of compound 48/80, and (b) to compare the effects of high dietary calcium intake in the rat on leg muscle and gastric tissue HM.

Our findings indicate a direct, *in vitro*, effect of compound 48/80 on rat, guinea pig, and chick leg muscle HDC activity but no similar direct effect on gastric tissue. High dietary Ca^{2+} was shown to stimulate gastric HDC activity in the rat but had no effect on muscle HDC. These results point to possible different control mechanisms in these two organs and to an additional role for compound 48/80, aside from its known function as an HM-releasing agent from mast cells.

Methods. *Animals, diets, and tissues.* Tissues for analysis were excised from male CD rats (125–160 g), male CD mice (20 g), both from Charles River Breeding Laboratories, Wilmington, Massachusetts, male Duncan–Hartley guinea pigs (4–5 months old, 350–400 g), and male, 2-week-old Leghorn chickens (85–110 g). The chickens were housed in groups and the other animals individually. Food and water were provided *ad libitum*. Commercial diets were used except for the calcium rat study; for this trial, the rats were fed either a normal or a high dietary calcium diet (0.45 or 3.25%, the additional 2.8% supplied as $CaCO_3$), as described previously (9). Tissues were either homogenized immediately or frozen at −20°C; all analyses were completed within 1 week of sacrifice.

Assays. Hind leg muscle tissue from all species was prepared for analysis by homogenization (Polytron, Brinkman Instruments) in 0.1 *M* sodium phosphate buffer, pH 7.4, with 0.1% Triton X-100 added, then centrifuged for 10 min at 4900*g* at 4°C. The supernatant liquid was decanted and used for the HDC

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and protein assays in the case of muscle tissue. The supernatant was then boiled for 5 min, recentrifuged under the same conditions and the supernatant liquid used for the HM assay (for both muscle and stomach). For stomach tissue, the HDC assay was carried out directly on the homogenate prior to centrifugation (see Results and Discussion for further elaboration). HDC assays were carried out by the method of Snyder and Epps (10) with the following modifications: the assay was run in 16 × 100-mm Borscillate test tubes (Fisher Scientific Co., Pittsburgh, Pa.) rather than in 10-ml Erlenmeyer flasks, and, where indicated, additional compounds were added to the assay cocktail. HM concentration was assayed by the method of Taylor and Snyder (11) with the modifications developed by Ishibashi *et al.* in our laboratory (12). Carnosinase (CASE) activity was determined by the method of Margolis and co-workers (13), and protein concentration of tissues and supernatants was determined by the Bio-Rad microprocedure (Bio-Rad, Rockville Center, N.Y.). Muscle and stomach protein content was approximately 18% of the wet tissue.

Histology. Leg muscle and stomach tissues were rinsed in sodium phosphate buffer (pH 7.4), followed by fixing in 4.0% formaldehyde overnight. The tissues were next dehydrated overnight in 70% ethanol, followed by 1 hr each in 85, 95, and twice with 100% ethanol. After dehydration, the tissues were cleared twice for 1 hr in 100% xylene and then embedded in paraffin. Finally, the tissues were sectioned, stained for 5 min with toluidine blue, and observed under light microscopy.

Results and Discussion. The first experiment was designed to compare HDC activity in tissue homogenate versus supernatant solution centrifuged at different speeds. The assay as originally reported (10) and as carried out in most laboratories uses tissue homogenates. We had noted greater ease, however, in using supernatant solutions instead of the complete homogenate. Recently, Wohl and Maslinski (14) reported carrying out the HDC assay on the supernatant solution from a rat stomach homogenate centrifuged for 5 min at 1800*g*.

The first experiment showed that, for rat muscle tissue, HDC activity in the supernatant solution peaked when it was prepared at 4900*g*. Supernatant HDC activity was significantly greater than homogenate activity (0.37 vs 0.31 nmol CO_2/mg Pr/hr). Muscle HM concentration was unaffected by centrifugation speed. In contrast, rat gastric HDC activity was significantly reduced in supernatant solutions resulting from centrifugation above 4900*g*. HM concentrations in the gastric tissue supernatant solutions were not significantly affected.

As a consequence of these findings, we have, in all subsequent experiments, assayed gastric tissue HDC activities in the homogenate, but muscle tissue HDC was assayed in supernatant solutions resulting from centrifugation at 4900*g* for 10 min at 4°C.

The next experiment was designed to study the effect of (a) *in vitro* addition of compound 48/80 to rat leg muscle and gastric tissue homogenate prior to the HDC assay,[3] and (b) supplementation of the diet with a high level of calcium for 7 days prior to sacrifice of the animals and assay for HDC activity and HM concentrations of muscle and gastric tissue.

Table I shows that dietary treatment with additional (2.8%) calcium had a highly significant effect in increasing HDC activity of stomach tissue as well as of histamine concentration. On the other hand, the high level of dietary calcium had no effect on either muscle HDC activity or histamine concentration. The effect noted here for gastric tissue confirms an earlier report from this laboratory (9), but demonstrates that muscle HDC activity is not affected by high dietary calcium intake.

The *in vitro* addition of compound 48/80 to the rat muscle homogenate significantly increased both HDC activity and histamine concentration. It had no effect, however, on stomach HDC activity or histamine concentration. The *in vitro* effect observed from 48/80 additions to muscle homogenate is similar, although of a lower magnitude, to the effect obtained from muscle when 48/80 was injected ip prior to sacrifice of the experimental rats (8). We are not aware of any other reports in which HDC activity was studied in muscle

[3] The homogenate was allowed to incubate with the 48/80 for 1 hr. Preliminary tests for up to 4 hr of incubation showed 1 hr to give a maximal response.

TABLE I. EFFECT OF DIETARY CALCIUM LEVEL AND *IN VITRO* TREATMENT WITH COMPOUND 48/80 ON RAT MUSCLE AND GASTRIC HDC ACTIVITY AND HM CONCENTRATION

Tissue	Dietary Ca (%)	HDC activity[a] (nmole CO_2/hr/mg Pr)		HM concentration[b] (nmole/g)	
		+48/80[c]	No 48/80	+48/80[a]	No 48/80
Muscle	0.45	$0.36 \pm 0.03^{d,f}$	0.29 ± 0.09^{g}	$58.31 \pm 9.42^{e,h}$	33.47 ± 4.22^{j}
	3.25	0.34 ± 0.04^{f}	0.27 ± 0.04^{g}	53.02 ± 6.24^{h}	31.09 ± 3.97^{i}
Stomach	0.45	0.83 ± 0.14^{j}	0.79 ± 0.18^{j}	82.42 ± 4.95^{l}	81.82 ± 6.65^{l}
	3.25	1.87 ± 0.21^{k}	1.75 ± 0.42^{k}	129.29 ± 11.38^{m}	141.52 ± 13.29^{m}

[a] Mean values ± standard deviation for 12 animals per group.
[b] Mean values ± standard deviation for 15 animals per group.
[c] 48/80 added directly to assay cocktail at a level of 5 mg/kg body wt and permitted to incubate for 1 hr at 37°C.
[d] Values with different superscripts are significantly different at $P \leq 0.05$.
[e] Values with different superscripts are significantly different at $P \leq 0.001$.

tissue upon *in vitro* addition of compound 48/80.

The third experiment was designed to test the species specificity for the direct action of compound 48/80 on HM concentration in leg muscle and gastric tissue. Table II gives the comparative results for rat, guinea pig, chicken, and mouse. An HM stimulatory effect for compound 48/80, albeit of a lower magnitude than for rat, was observed for guinea pig and chicken, but not for mouse leg muscle. No stimulation was observed in gastric tissue of any of the species studied.

The histological observations of muscle and gastric tissues stained with toluidine blue (for mast cells) indicated that mast cells are present in the loose connective tissue portion of muscle tissue between muscle fibers. The mast cells appear irregularly and represent only a small fraction of the total muscle fiber cells. Further, the concentration of mast cells in muscle tissue from rat, mouse, and guinea pig was closely similar.

By comparison, a large population of mast cells was present in the gastric tissue of all species studied. These cells appeared in a relatively uniform concentration throughout the connective tissue layer of the stomach. A cell

TABLE II. COMPARATIVE EFFECT OF *IN VITRO* TREATMENT WITH COMPOUND 48/80 ON MUSCLE AND GASTRIC HM CONCENTRATION OF RAT, GUINEA PIG, CHICKEN, AND MOUSE

	Tissue HM[a] (nmole/g)			
	Stomach		Muscle	
	+48/80[b]	No 48/80	+48/80	No 48/80
Guinea pig[c]	$62.52 \pm 3.84^{d,g}$	64.27 ± 4.19^{g}	35.89 ± 1.89^{h}	32.84 ± 1.48^{i}
Chicken[e]	79.15 ± 5.26^{g}	80.05 ± 6.89^{g}	34.24 ± 2.07^{h}	31.26 ± 3.63^{i}
Mouse[e]	ND	ND	24.37 ± 2.62^{h}	22.98 ± 2.62^{h}
Rat[f]	89.29 ± 6.27^{g}	91.34 ± 8.61^{g}	58.31 ± 9.42^{h}	33.47 ± 4.22^{i}

Note. ND = not determined.
[a] For stomach, the HM assay was carried out directly on homogenate; for muscle, the homogenate was centrifuged for 10 min at 4900*g* and the supernatant solution was used in the HM assay.
[b] 48/80 added directly to assay cocktail at a level of 5 mg/kg body wt and permitted to incubate for 1 hr at 37°C.
[c] Mean values ± standard deviation for 10 animals per group.
[d] Values within each row with different superscripts are significantly different at $p \leq 0.05$.
[e] Mean values ± standard deviations for 12 animals per group.
[f] Mean values ± standard deviations for 15 animals per group.

TABLE III. EFFECT OF *IN VITRO* ADDITION OF $CaCl_2$ ON HDC ACTIVITY AND HM CONCENTRATION OF RAT MUSCLE AND STOMACH FROM RATS FED HIGH OR NORMAL DIETARY CALCIUM LEVELS

Tissue	Dietary Ca (%)	HDC activity[a] (nmole CO_2/hr/mg Pr)		HM concentration[b] (nmole/g)	
		+$CaCl_2$[c]	No $CaCl_2$	+$CaCl_2$[c]	No $CaCl_2$
Muscle	0.45	0.22 ± 0.04[d,f]	0.24 ± 0.10[f]	32.04 ± 2.58[e,g]	31.89 ± 3.62[g]
	3.25	0.20 ± 0.05[e]	0.28 ± 0.07[f]	34.07 ± 4.06[g]	29.07 ± 4.21[g]
Stomach	0.45	1.01 ± 0.10[h]	0.90 ± 0.07[i]	83.81 ± 5.02[j]	80.63 ± 4.98[j]
	3.25	1.82 ± 0.23[l]	1.74 ± 0.24[l]	122.43 ± 10.62[k]	124.54 ± 8.35[k]

[a] Mean values ± standard deviation for eight animals per group.
[b] Mean values ± standard deviation for nine animals per group.
[c] 2.5 μM Ca^{2+} added to assay cocktail.
[d] Values with different superscripts are significantly different at $P \leq 0.05$.
[e] Values with different superscripts are significantly different at $P \leq 0.001$.

count indicated an approximate ratio of mast cells in gastric tissue to muscle tissue of 12 to 1.

The relatively small number of mast cells in muscle suggests that the histamine-stimulatory effect of compound 48/80 in muscle tissue cannot be due simply to a release of histamine from mast cells. If this were a factor, 48/80 addition to gastric tissue homogenates should have promoted a much greater rise in HDC activity and HM, when, in fact, it gave no response at all.

In the fourth experiment, we studied the effect of *in vitro* addition of extra calcium (2.5 μM Ca^{2+} per assay tube) on HDC activity and HM concentration. There was a 10% significant increase (Table III) in gastric HDC activity of rats fed the normal (0.45%) level of dietary calcium. This increased activity, however, was not translated into a higher concentration of gastric HM. As in Experiment 2, (Table I), the high dietary calcium regime produced highly significant increases in gastric HDC activity and HM concentrations.

The fifth experiment was designed to ascertain if compound 48/80 affected a purified bacterial (*Escherichia coli*) HDC preparation. Compound 48/80 was added to the assay medium which contained the bacterial HDC with or without muscle or gastric tissue. Table IV shows that 48/80 again (as in Experiment 2, Table I) significantly increased HDC activity in muscle, but had no effect on stomach or on the bacterial preparation alone or in combination with the rat tissues.

The final experiment in this series was a study of the *in vitro* addition of compound 48/80 to muscle in relation to CASE activity. We had previously shown in muscle that *in vivo* ip injection of compound 48/80 increased CASE activity significantly later than it exerted its effect on HDC activity (8). This timing is important since CASE releases histidine (HIS) for HM synthesis. In the current experiment (Table V), however, compound 48/80, when added *in vitro,* had no effect on CASE activity, suggesting that the earlier observed *in vivo* increase was indirectly caused by the need for

TABLE IV. EFFECT OF *IN VITRO* ADDITION OF COMPOUND 48/80 TO BACTERIAL HDC PREPARATIONS

HDC preparation	HDC activity[a] (nmole CO_2/hr/mg Pr)	
	+48/80[b]	No 48/80
Muscle	0.36 ± 0.08[c,d]	0.28 ± 0.05[e]
Bacterial	15.07 ± 0.92[f]	15.18 ± 1.44[f]
Muscle + bacterial	16.82 ± 1.08[f]	16.51 ± 1.82[f]
Stomach	0.81 ± 0.17[g]	0.83 ± 0.10[g]
Stomach + bacterial	15.94 ± 1.99[f]	15.01 ± 2.53[f]

[a] Mean values ± standard deviation for 12 animals per group.
[b] 48/80 added directly to assay cocktail at a level of 5 mg/kg body wt and permitted to incubate for 1 h.
[c] Values within each row with different superscripts are significantly different at $P \leq 0.05$.

TABLE V. EFFECT OF *IN VITRO* ADDITION OF 48/80 ON RAT MUSCLE CASE ACTIVITY

48/80 addition[b]	CASE activity[a] ($\times 10^{-2}$ nmole/hr/mg Pr)
+	5.31 ± 1.24[c,d]
−	4.82 ± 1.88[d]

[a] Mean values ± standard deviation for 15 animals per group.

[b] 48/80 was added directly to the assay cocktail at 5 mg/kg body wt at time of sacrifice. Incubation was for 1 hr.

[c] Values with different superscripts are significantly different at $P \leq 0.05$.

more HIS in response to the increased HDC activity and the increased amount of HM formed.

General Comments. The present study clearly shows that the HDC from different tissues (muscle and stomach) reacts differently to different stimuli. Compound 48/80 directly (*in vitro*) stimulates muscle HDC activity, but has no effect on stomach or bacterial HDC. A high dietary level of calcium doubles gastric HDC activity, but is completely inactive vis a vis muscle HDC.

Since it is highly unlikely that *de novo* protein synthesis is occurring in the *in vitro* muscle homogenate system, 48/80 might be exerting its effect by removing (binding?) an HDC inhibitor present in the muscle. It is also very unlikely that the 48/80 effect in muscle is due to mast cell histamine release which, in turn, might stimulate HDC activity, since histological studies indicated few mast cells in muscle, particularly in comparison with gastric tissue.

The finding that compound 48/80 stimulates HM synthesis also confirms a recent report by Rothschild and Fortunato (15) who reported the stimulation of HM synthesis in rat peritoneal fluid cells. These authors suggest that the HM synthesizing function of compound 48/80 has been overlooked in the past because it occurs relatively long after the maximal HM release has occurred.

The differential response noted here for muscle and gastric HDC is in line with the finding of Yamada *et al.* (16) that there were two distinct types of HDC, one present in stomach and the other in brain. Savany and Cronenberger (17) further suggested that there are three separate forms of HDC present in rat stomach. The present studies suggest that the muscle form of HDC may also be distinct from the gastric (and brain) form, a finding which had not been previously demonstrated. The differential response of muscle, stomach, and bacterial HDC to compound 48/80 suggests that there may be inhibitory factors present in muscle supernatant solution that are absent from the stomach and bacterial systems and which compound 48/80 inactivates or binds.

The lack of a response on muscle CASE activity from *in vitro* addition of 48/80 (in contrast to an *in vivo* response and to the previously noted *in vitro* stimulation of muscle HDC) may be interpreted as further evidence for the hypothesis advanced from this laboratory (8) that CASE stimulation (*in vivo*) is related to and dependent upon the depletion of free histidine for histamine synthesis, followed by repletion from degraded carnosine.

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