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Mesenteric Mast Cell Degranulation is Not Essential For Conditioned Taste Aversion[1]

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PERSINGER, M. A. AND T. B. FISS. *Mesenteric mast cell degranulation is not essential for conditioned taste aversion.* PHARMAC. BIOCHEM. BEHAV. 9(6) 725–730, 1978.—The possible role of mesenteric mast cell degranulation as the mediator of the initial UCS effects in the complex sequences leading to conditioned taste aversion (CTA) was studied. Both LiCl and Compound 48/80, a potent mast cell degranulator, produced CTA to 10% sucrose. Whereas the Compound 48/80 groups displayed massive mast cell degranulation, neither the LiCl treated nor saline control groups demonstrated any histologically determinable alterations. Administration of the antihistamine chlorpheniramine at dosages known to block radiation-induced CTA before the sucrose CS-UCS pairings did not block either LiCl- or Compound 48/80-induced CTA; the antihistamine actually facilitated the aversion. However, pretreatment with the antihistamine did not alter mesenteric mast cell morphology.

Compound 48/80 Mast cells Mesentery Conditioned taste aversion Rat Lithium chloride Antihistamine

THE CELLULAR mediator of conditioned taste aversion (CTA) between intraperitoneal injections or whole body applications (ionizing radiation) of the UCS and the actual CNS process is not clear. Garcia [6] speculated that CTA inducing agents may all produce some general visceral malaise or illness. Although many CTA inducing agents, such as cyclophosphamide, apomorphine and lithium chloride (LiCl) have been reported to evoke overt symptoms of "sickness," CTA-eliciting doses of ionizing radiation do not appear to be associated with clear signs of sickness [6]. Other drugs, such as sodium pentobarbital do not produce obvious signs of toxicity in rats but still elicit CTA [1].

The cellular mediator of CTA following abdominal introduction of appropriate stimuli should display a graded capacity of response to the variety of CTA agents such that "sickness" is more correlative to secondary factors rather than the source of the effect. One possible candidate for the cytological mediator of CTA agents is the mast cell (MC), specifically mesenteric mast cells. Since the mesentery carries blood vessels, lymphatics and nerves to (and from) visceral organs, MCs within this matrix are in a strategic position to influence a variety of physiological chemical reactions.

MCs contain a storehouse of biopotent materials including histamine, heparin, proteinpolysaccharides, polypeptides, serotonin, and dopamine [10]. These substances are contained within cytoplasmic granules that can be released in an explosive or in a gradual manner by either a small portion or all of a MC population following contact with a vast number of substances including ionizing radiation, many of the CTA chemicals and even distilled water [11]. Once released into interstitial fluids, MC compounds can contribute to local vasodilation, hematological changes, initiation of tissue leukocytic activity and immune-related responses [10]. MCs are more well known at a systemic or organismic level for their contribution to anaphylactic shock.

Several lines of evidence imply the contribution of MC degranulation to CTA: (1) Agents known to elicit CTA are either established or likely peritoneal/mesenteric MC degranulators [11]. (2) Ionizing radiation, a frequently used UCS in CTA studies [6,8] initiates massive degranulation in abdominal MC populations [11]. Antihistamines can not only prevent radiation-induced MC degranulation [12] but also block CTA to saccharin paired with ionizing radiation [8]. (3) ACTH and related structures can be degranulators of abdominal MCs [11]; plasma corticosterone levels have been demonstrated to be indices of the strength of CTA [13]. (4) The rat which has been called a mast cell animal [4] because of the large concentrations of these cells throughout the connective tissue matrix, is especially prone to CTA paradigms, e.g., bait shyness.

To test the MC hypothesis, experiments were designed to answer the following predictions. If mesenteric MCs are associated with CTA, then the pairing of the CS sucrose with the UCS Compound 48/80 [11], a well known MC degranulator, should produce CTA to sucrose comparable to usual experimental dosages of LiCl or ionizing radiation. Pretreatment with the antihistamine chlorpheniramine

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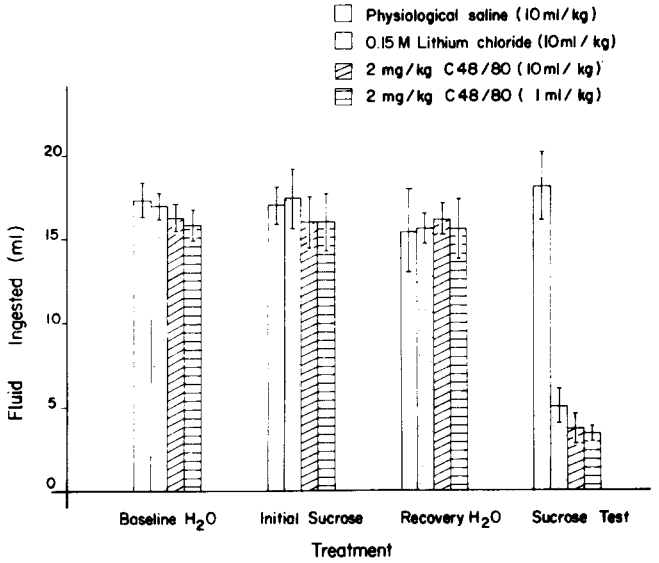

FIG. 1. Mean water consumption during baseline and recovery days and mean 10% (w/v) sucrose consumption during initial sucrose and sucrose test days for groups of rats that were injected after the first sucrose presentation with either physiological saline, 0.15 M lithium chloride, or one of two volumes of Compound 48/80. Vertical bars indicate standard errors of the mean.

maleate at dosages (20 mg/kg) sufficient to block radiation induced aversion to saccharin (8) should block CTA to sucrose induced by C 48/80 but not by LiCl (replication of [8]). (Implicitly, this argument demands that some dosage or some type of antihistamine should be able ultimately to block LiCl-induced CTA.) If MC degranulation contributes to CTA in general, then the differential effects of the antihistamine upon the CTA producing properties of different UCSs should reflect the degree of preventative degranulation. Consequently, both LiCl-induced and C 48/80-induced CTA should be associated with MC degranulation while pretreatment with chlorpheniramine should prevent CTA to C 48/80 as well as MC degranulation but not eliminate CTA or degranulation to LiCl.

EXPERIMENT 1

METHOD

In two separate test runs, 28 naive male 300 day old (600 ± 50 g) albino Wistar rats were removed from group housing, placed in single cages, maintained on a 23.7 hr water deprivation schedule and exposed to a typical CTA paradigm [9]. For 5 days, they were given access to water for 20 min per day in their home cages. On Day 6 (conditioning day), the rats were allowed to drink 10% (w/v) sucrose for 20 min and then randomly allocated to one of four groups (n=7 each). Within 5 to 15 min, the rats were injected IP with one of four substances: (1) 10 cc/kg of 0.15 M LiCl, (2) 10 cc/kg of 2 mg/10 cc of C 48/80 in physiological saline, (3) 1 cc/kg of 2 mg/cc of C 48/80 in physiological saline, or (4) 10 cc/kg of physiological saline. Due to injection difficulties, one rat in the physiological saline group was eliminated from the study. On Days 7 and 8 (recovery days), the rats were allowed water only for 20 min per day. On Day 9 (test day), rats were allowed 20 min access to the 10% sucrose solutions. All solutions were delivered in 1 ml graduated 100 ml drinking bottles. The following colony conditions existed for all experiments: LD cycle: 12:12; temperature: 21 ± 1°C; relative humidity: 45 ± 5%; background noise: 70 ± 2 dB. All data analyses were completed by computer using SPSS packages; sample analysis were checked manually.

RESULTS

The means and standard errors for water intake (in ml) during baseline and recovery days and for sucrose intakes (in ml) during conditioning and test days are shown in Fig. 1. One way analyses of variance between groups demonstrated no significant differences ($F<1$, $p>0.05$) in fluid consumption during baseline, conditioning or recovery days. However, highly significant ($F(3,23)=34.66$, $p<0.001$) differences existed between groups for sucrose consumption on the test day. A posteriori tests (Duncan's and Scheffe's set at $p<0.05$) indicated that the LiCl and C 48/80 groups drank significantly less sucrose than the physiological saline controls. There were no significant differences between C 48/80 and LiCl groups.

EXPERIMENT 2

Although intense CTA comparable to LiCl injections was induced by C 48/80, some animals demonstrated adverse post-injection malaise and respiratory complications. Consequently, this study was instituted to determine optimal CTA-C 48/80 dosages with younger animals.

METHOD

Experimentally naive 70–80 day old (325 ± 20 g) male albino, Wistar rats were obtained from Bio Breeding Laboratories (Ottawa) and maintained in automatic cages for one to two weeks on food and water ad lib. In two separate test runs, 24 and 30 rats were removed from group housing, placed in single cages, maintained on a 23.7 hr water deprivation schedule, and exposed to the CTA paradigm. On the conditioning day, within 5 to 50 min after termination of sucrose availability, the rats were injected (IP) with either: (1) 2 cc/kg of physiological saline, (2) 10 cc/kg of 0.15 M LiCl, (3) 2 mg/kg C 48/80, (4) 1 mg/kg C 48/80, (5) 0.6 mg/kg C 48/80, (6) 0.2 mg/kg C 48/80 or (7) 0.02 mg/kg C 48/80 volumes were 2 cc/kg). An eighth group served as non-handled controls. Since 6 of the total number of rats injected with the 2 mg/kg dosage of C 48/80 died within 1 hr (4 in sequence 1 and 2 in sequence 2), extra rats were added to this group in the second part of the experiment. On Day 9 (test day), following the two typical recovery days, rats were allowed 20 min access to the 10% sucrose solutions. Since absolute sucrose consumption values were comparable to Experiment 1, sucrose preference scores were derived for brevity. Sucrose preference scores were calculated by dividing the amount of sucrose solution consumed on the test day by the amount consumed on the conditioning day.

RESULTS

Mean sucrose preference scores for the eight groups are shown in Fig. 2. As can be seen, the most apparent CTA was associated with the LiCl injections and the 2.0 mg/kg C 48/80 injections; as mentioned 6 of the 12 rats in the latter group died. A one-way analysis of variance (ANOVA) between the

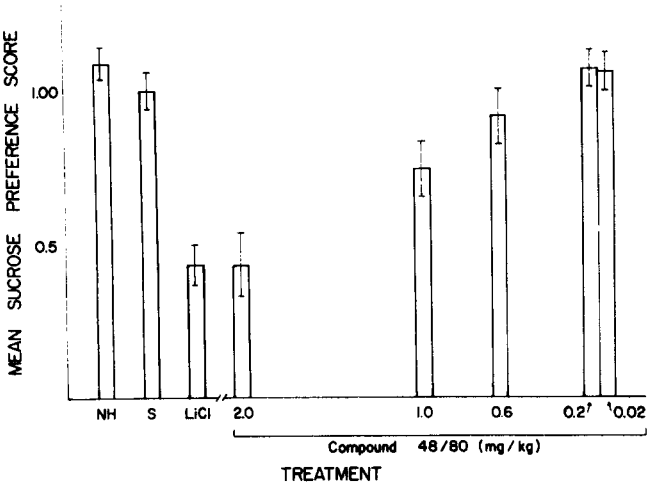

FIG. 2. Mean sucrose preference scores for groups (n=6/group) of rats given different dosages of Compound 48/80 (in 2 cc/kg volumes), 10 cc/kg of 0.15 M LiCl, physiological saline (2 cc/kg): S, or no treatment (non-handled: NH). Vertical bars indicate standard errors of the mean.

groups was significant, $F(7,40)=11.97$, $p<0.001$. Duncan's multiple range tests set at $p<0.05$ demonstrated that the LiCl and 2 mg/kg C 48/80 groups were different from all other groups and that the 1 mg/kg C 48/80 group was significantly different from both the former two groups and the other groups. All other group differences were not significant statistically.

EXPERIMENT 3

On the basis of the above results, the 1 mg/kg dosage of C 48/80 was selected to test the CTA-mast cell hypothesis. This dosage still produced significant taste aversion, but without the morbidity associated with the 2 mg/kg dosage. In order to test the possible prophylactic effects of chlorphineramine toward conditioned sucrose taste aversion, this substance was initially injected into 60–70 day old male Wistar rats one hr *before* sucrose availability on the conditioning day. However, we found that the 12 rats that had been injected with the antihistamine (20 mg/kg) volumes consumed only 11.3 ± 4.9 ml of sucrose compared to the saline-injected controls, 22.9 ± 3.8 ml. Since the differential consumption of the CS was a clear confounding variable [2,3], this experiment was terminated. We decided to inject the antihistamine after the initial sucrose consumption but before the LiCl, C 48/80 or saline pairings.

METHOD

Experimentally naive 70–80 day old (320 ± 25 g) male Wistar rats were exposed to the paradigm until the conditioning day. Within 30 min after termination of sucrose availability, 12 rats were injected IP with 20 mg/kg (2 cc/kg) of chlorpheniramine maleate [8] while another 12 rats were injected with physiological saline (2 cc/kg). One hr later, equal numbers of rats from each of the two pretreatment groups were injected IP with either LiCl (10 cc/kg), 1 mg/kg C 48/80 (2 cc/kg) or physiological saline (2 cc/kg). Following the typical two day recovery period, sucrose solutions were again presented on the test day.

RESULTS

The means and standard deviations for group sucrose preference scores are presented in Table 1. A two-way ANOVA demonstrated a significant pretreatment difference, $F(1,18)=5.72$, $p<0.05$; the antihistamine appeared to enhance the CTA. However, there were neither significant treatment (LiCl, C 48/80 or saline), $F(2,18)=2.24$, ns, nor treatment by pretreatment interactions, $F(2,18)=2.54$, ns.

EXPERIMENT 4

Chlorpheniramine at dosages sufficient to block CTA to saccharin when injected before presentation of ionizing radiation did not prevent CTA when sucrose was paired with either LiCl (a replication of [8]) or C 48/80. However, in the Levy [8] study, only a single injection (the antihistamine) was involved (since the UCS was hard core radiation) while in Experiment 3, the two separate injections of pretreatment and treatment appeared to attenuate the CTA for both LiCl and C 48/80 rats compared to the results of Experiments 1 and 2. Since a period of about 1 hr elapsed between the two injections for each rat, the possibility of some compounding antagonizing effect from this procedure existed. Similar observations were made by Domjan and Best [5]. Consequent-

Table 1

MEANS AND STANDARD DEVIATIONS (±) OF SUCROSE PREFERENCE SCORES FOR RATS THAT WERE PRETREATED WITH EITHER CHLORPHENIRAMINE (20 MG/KG), OR PHYSIOLOGICAL SALINE AND THEN TREATED 1 HR LATER WITH EITHER 0.15M LICL (10 CC/KG), COMPOUND 48/80 (1 MG/KG) OR PHYSIOLOGICAL SALINE (2 CC/KG) IN EXPERIMENT 3

Pretreatment	Saline	Treatment LiCl	C 48/80	Combined Pretreatment (n=12/group)
Saline	1.06 ± 0.08[b]	0.84 ± 0.06	0.72 ± 0.08	0.87 ± 0.16[a]
Antihistamine	0.69 ± 0.20[a]	0.75 ± 0.22	0.69 ± 0.23	0.71 ± 0.20[b]
Combined Treatment (n=8/group)	0.88 ± 0.24	0.80 ± 0.15	0.70 ± 0.16	

a vs b $p<0.05$.

TABLE 2

MEANS AND STANDARD DEVIATIONS (±) OF SUCROSE PREFERENCE SCORES FOR RATS THAT WERE PRETREATED WITH EITHER CHLORPHENIRAMINE (20 MG/KG) OR PHYSIOLOGICAL SALINE AND THEN TREATED WITHIN 30 SEC WITH EITHER 0.15M LICL (10 CC/KG), COMPOUND 48/80 (1 MG/KG) OR PHYSIOLGICAL SALINE (2 CC/KG) IN EXPERIMENT 4

Pretreatment	Treatment Saline	Treatment LiCl	Treatment C 48/80	Combined Pretreatment (n=24/group)
Saline	0.88 ± 0.26	0.72 ± 0.14	1.00 ± 0.30	0.87 ± 0.26[a]
Antihistamine	0.76 ± 0.13	0.57 ± 0.19	0.61 ± 0.16	0.65 ± 0.18[b]
Combined Treatment (n=16/group)	0.82 ± 0.21[a]	0.64 ± 0.18[b]	0.81 ± 0.31	

a vs b $p<0.05$.

ly, in Experiment 4, rats were given the two injections (pretreatment and treatment) within 30 sec. Since the crucial support of the mast cell hypothesis was clearly contingent upon cytological alterations, rather than pharmacological inference, histological analyses of mesenteric mast cells were completed.

METHOD

In two separate sequences, a total of 48 male 60–70 day old (290 ± 25 g) naive Wistar rats were exposed to the usual CTA paradigm until the conditioning day. Within 50 min after initial sucrose consumption, rats were injected IP separately with either: (1) 20 mg/kg of chlorpheniramine (2 cc/kg) and then either 0.15 M LiCl (10 cc/kg), 1 mg/kg C 48/80 (2 cc/kg) or physiological saline (2 cc/kg) or (2) physiological saline (2 cc/kg) and then either the LiCl, saline or C 48/80 solutions. The time between the two injections was 30 sec. Following the two days of recovery, the rats were tested for sucrose consumption. Within 1 hr of the test period, 6 rats from each of the 6 groups were taken quickly to the laboratory and decapitated. For each rat, a section of mesentery containing moderate sized blood vessels was carefully placed (without excessive stretching) over the mouth (6.5 cm^2) of a test tube, maintained in position by tying thread around the test tube lip and quickly fixed in E.F.A. (90 pts 80% ethanol, 5 pts. 30% formaldehyde and 5 pts. glacial acetic acid). The mesenteric sections for each group were selected from the entire field of small intestine in order to increase sampling range. Following dehydration, clearing and paraffin embedding, the tissues were microtomed at 6 μm and then stained with toluidine blue 0 for mast cells [7].

Mast cells from two slides for each rat were evaluated at 100 × and 400 × magnification according to the following scale: (1) normal nuclei, clear and copious metachromatic granules in cytoplasm; (2) characteristics of 1 except granules apparent immediately adjacent to outside cell membrane (typical of procedural artifacts, e.g., excessive mechanical stimulation; (3) characteristics of 2 but less cytoplasmic granules; (4) characteristics of 3 but only half the number of granules and reduction in cytoplasmic volumes; (5) cell shrinkage, low density granules, globulation of cytoplasmic metachromasia, but some normal metachromatic granules in at least 50% of the cells; (6) low density granules, cell shrinkage, excessive cytoplasmic globulation in all cells and poorly defined cells; (7) entire population of poorly defined cells, no metachromasia in cell but metachromatic granules dispersed over wide area, i.e., more than 5 cell radii, (7) is typical of actue C 48/80 changes within 1–2 hr after a single 1 mg/kg injection. This scale represents the usual range displayed by MC populations following administration of "low to high degranulating" drugs in this laboratory. The average of the two slides for each rat was used as its score for group analysis.

RESULTS

The means and standard deviations of the sucrose preference scores for the six groups are shown in Table 2. A two-way ANOVA demonstrated again a significant enhancement of CTA in the rats pretreated with the antihistamine relative to the rats pretreated with saline, $F(1,42)=14.16$, $p<0.001$. Significant differences existed between the three treatments (LiCl, C 48/80 and saline, $F(2,42)=3.64$, $p<0.05$, but not for the interaction, $F(2,14)=2.14$, $p>0.05$. Ad hoc Duncan's multiple range tests set at $p<0.05$ indicated that LiCl groups displayed lower preference scores (greater CTA) than the saline or C 48/80 groups; there were no significant differences between saline or C 48/80 groups.

Histological results were clear: neither LiCl induced CTA nor pretreatment with antihistamine detectably altered MC architecture. As can be seen in Fig. 3 (C,F), only compound 48/80 (1 mg/kg), regardless of pretreatment, produced profound changes in the size and cytoplasmic granulation of mesenteric MCs. This reduction in cell widths and degree of cytoplasmic granulation (but maintenance of normal sized nuclei) is typical of MCs 2–3 days following a single injection of 1 to 2 mg/kg of C 48/80 (unpublished parametric studies). Both antihistamine or saline pretreated and LiCl or saline treated rats displayed normal mesenteric MC profiles (Fig. 2A, B, D, E). The gross differences between main treatments were clearly evident in the histological rating (1–7) scale (Table 3.) Although MC numbers were not quantitatively determined, there were no gross numerical differences between LiCl or saline groups from either pretreatment condition. However, whereas more than 90% of all MCs in any given section of mesentery from C 48/80 injected rats showed the

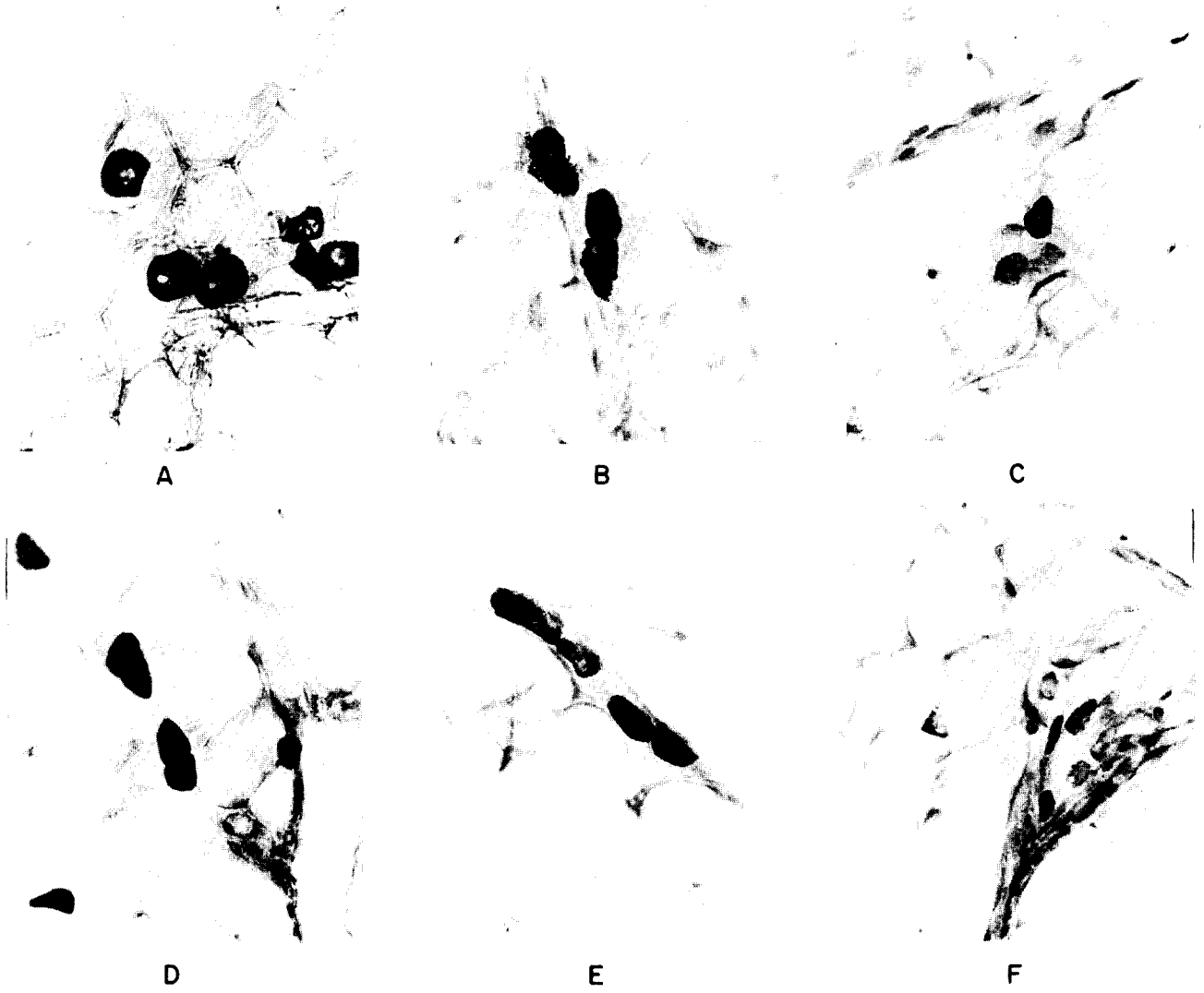

FIG. 3. Photomicrographs of representative mast cells from mesenteries of rats that had been either pretreated with physiological saline and treated with saline (A), LiCl (B) or compound 48/80 (C) or pretreated with antihistamine and then treated with saline (D), LiCl (E) or Compound 48/80 (F). 400×magnification; toluidine blue 0.

TABLE 3

MEANS AND STANDARD DEVIATIONS OF HISTOLOGICAL SCORES FOR THE DEGREE OF MESENTERIC MAST CELL DEGRANULATION FROM 36 RATS THAT HAD BEEN PRETREATED WITH EITHER CHLORPHENIRAMINE (20 MG/KG) OR PHYSIOLOGICAL SALINE AND THEN TREATED WITH EITHER LICL (10 CC/KG), COMPOUND 48/80 (1 MG/KG) OR SALINE (2 CC/KG), (N=6/GROUP)

Pretreatment	Treatment		
	Saline	LiCl	C 48/80
Saline	1.5 ± 0.5	1.4 ± 0.4	5.2 ± 0.8
Antihistamine	1.4 ± 0.2	1.6 ± 0.4	5.5 ± 0.7
Total	1.4 ± 0.3[a]	1.5 ± 0.4[a]	5.3 ± 0.7[c]

a vs c $p<0.001$.

gross alteration, not a single MC in any of the mesenteries from LiCl or saline rats showed this extreme profile.

DISCUSSION

Neither the pharmacological nor the histological data supported the hypothesis that mesenteric mast cell degranulation is the primary cytological correlate of CTA-producing UCSs. LiCl-injected rats demonstrated CTA to the sucrose solution but showed no apparent MC degranulation that was still evident on the test (and kill) day; the intensity of the CTA was quite variable. Antihistamine injections before either saline, LiCl or C 48/80 did not alter MC degranulation although they did *increase* the overall intensity of the CTA.

The antihistamine chlorpheniramine (20 mg/kg) administered 1 hr or 30 sec before LiCl did not attenuate the CTA, a result similar to Levy *et al.* [8] who used saccharin instead of sucrose as the CS. However, since LiCl did not degranulate the MCs whereas ionizing radiation presumably does [11], it was possible that the MC hypothesis could still be valid (for at least some kinds of CTA) if the antihistamine could prevent degranulation. In this study, the same dosage of chlorpheniramine used to block CTA induced by ionizing radiation [8] did not prevent the degranulation of MCs following C 48/80 injection. No doubt an optimal dosage argument still could be used since the relationship between MC degranulation and antihistamine dosage can be non-linear [11].

In addition to the failure for chlorphineramine to block LiCl-induced CTA [8], this study also replicates the CTA attenuating effects of two UCSs presented in close temporal proximity [5]. CTA was strongly displayed in both Experiments 1 and 2 where single LiCl or appropriate C 48/80 injections were made. However, in Experiments 3 and 4, where either saline or antihistamine were injected 1 hr or 30 sec before these UCSs, the CTA was markedly reduced or even eliminated.

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