Capsaicin Pre- and Post-Treatment on Rat Monocrotaline Pneumotoxicity

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Abstract

Monocrotaline (MCT) produces respiratory dysfunction, pulmonary hypertension (PH), and right ventricular hypertrophy (RVH) in rats. Tachykinins, such as substance P (SP) and neurokinin A (NKA), may mediate these effects. The purpose of this study was to investigate the length of tachykinin depletion (via capsaicin treatment) is needed to prevent (or attenuate) PH and/or RVH. Six groups of rats were injected subcutaneously with saline (3 ml/kg); capsaicin followed by saline or MCT (60 mg/kg); or MCT followed 7, 11, or 14 days later by capsaicin. Capsaicin (cumulative dose, 500 mg/kg) was given over a period of 4-5 days. Respiratory function, pulmonary vascular parameters, lung tachykinin levels, and tracheal neutral endopeptidase (NEP) activity were measured 21 days after MCT or saline injection. Capsaicin significantly decreased lung levels of SP but not NKA. Both capsaicin pretreatment and posttreatment blocked the following MCT-induced alterations: increases in lung SP and airway constriction; decreases in tracheal NEP activity and dynamic respiratory compliance. Administration of capsaicin before or 7 days after MCT blocked MCT-induced PH and RVH. The above data suggest that the early tachykinin-mediated airway dysfunction requires only transient elevated tachykinins, while progression of late tachykinin-mediated effects (PH and RVH) requires elevated tachykinins for more than one week.

Key Words: monocrotaline, pulmonary function, pulmonary hypertension, tachykinins

Introduction

Monocrotaline (MCT), a plant alkaloid from *Crotalaria spectabilis*, produces progressive pneumotoxic effects in the rat. MCT has both early effects (primarily on the airways) and late effects (primarily on the pulmonary vasculature and heart). A single subcutaneous injection of MCT causes lung edema, detected within 4 hours (31), followed by decreased lung volumes (7) and structural changes in the lung (25). Right ventricular hypertrophy and increased pulmonary arterial pressure have been seen within 3 weeks after MCT injection (28). MCT has also been shown to increase the level of substance P (SP) in lung tissue as early as 1 week after administration (33). Tachykinins [mainly SP and neurokinin A (NKA) in mammals (26)] can be released from afferent C-fibers (26); these neuropeptides not only can cause bronchoconstriction (15, 24, 30), but also have vascular effects. The primary enzyme responsible for degradation of tachykinins *in vivo* is neutral endopeptidase (NEP) (2); MCT has been shown to decrease airway NEP activity (33). Pretreatment of rats with capsaicin, resulting in depletion of tachykinins due to degeneration of afferent C-fibers (12), has been shown to attenuate many effects of MCT, including lung stiffness, airway constriction, pulmonary hypertension, and right ventricular

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hypertrophy (33). These data suggest that some of the effects of MCT are mediated by tachykinins. It is not known, however, how long the increased tachykinin levels must be present for these effects to occur. The purpose of this study was to examine the time course of capsaicin-modulated, MCT-induced respiratory dysfunction and pulmonary hypertension. Capsaicin was administered to deplete tachykinins either before or 7 to 14 days after MCT injection. It was hypothesized that the early effects of MCT (respiratory dysfunction) would require the presence of increased tachykinins for only a brief period of time, while the later effects of MCT (pulmonary hypertension and right ventricular hypertrophy) would be dependent on the presence of elevated tachykinins for a longer period.

Materials and Methods

Animal Treatments

Forty-seven male Sprague-Dawley rats weighing 150-200 g at the start of the study were randomly placed into 6 groups: control (n=8); capsaicin (n=8); capsaicin+MCT (n=8); MCT+capsaicinl (n=8); MCT+capsaicin2 (n=7); and MCT+capsaicin3 (n=8). All saline, capsaicin, and MCT injections were given subcutaneously. The rats in the control group were injected with 3 ml/kg of physiological saline 3 weeks before testing of respiratory function. The rats in the capsaicin group were given a total of 500 mg/kg of capsaicin over 4 consecutive days. The daily doses were 50, 100, 150, and 200 mg/kg, divided into 2 - 4 injections each day. The day after the last capsaicin injection, 3 weeks before testing of respiratory function, the rats were given 3 ml/kg of saline. The rats in the capsaicin+MCT group were given capsaicin as in the capsaicin group; they were then given 60 mg/kg of MCT one day after the capsaicin injections were completed. The rats in the MCT+capsaicin1, MCT+capsaicin2, and MCT+ capsaicin3 groups were injected with 60 mg/kg of MCT 3 weeks before testing of respiratory function. Seven days after MCT administration, the rats in the MCT+capsaicinl group were given a total of 500 mg/kg of capsaicin over a 5 day period. The rats in the MCT+capsaicin2 and MCT+capsaicin3 groups were given a total of 500 mg/kg of capsaicin (over a 4 day period as in the capsaicin group) 11 or 14 days, respectively, after MCT administration.

Functional Testing

On the twenty-first day after MCT or saline administration, the rats were anesthetized by intraperitoneal injection of 1.5- 2.0 g/kg of urethane, and the trachea and jugular veins were cannulated. The respiratory function of the rats was tested as described previously (32). Briefly, the anesthetized animal was placed in a 2.8-L whole-body plethysmograph. The animals were paralyzed by intravenous injection of 10 mg/kg of gallamine trichloride. They were immediately placed on artificial ventilation at a rate of 60 strokes per min and a tidal volume of approximately 2 ml. Airway opening (Pao) and pulmonary arterial pressures were detected with Cobe transducers. Flow was obtained from the pressure difference detected by a Statham PMl5 transducer across three 325-mesh screens in the wall of the plethysmograph. Pressure transducers were connected to a Grass Model 7D polygraph, which was connected to a Buxco Model 6 Pulmonary Mechanics Analyzer. Volume was calculated by integration of flow. Flow, volume, and pressure measurements were recorded on the polygraph. To perform the maximal expiratory flow-volume maneuver, the lungs were inflated to an airway opening pressure of 25-30 cm H_2O three times; the third time was via a solenoid valve connected to a pressure reservoir adjusted to provide a flow rate of 2.5-5 ml/sec. As soon as the Pao reached 30 cm H_2O, the inspiratory valve was closed and another solenoid valve, connected to a vacuum reservoir (-40 cm H_2O) was opened to induce maximal expiration. The resultant maximal expiratory flow-volume curve was plotted and stored on an oscilloscope. The functional residual capacity (FRC) was determined using a neon dilution method (13). The maximal expiratory flow-volume and pressure-volume curevs were used to obtain the maximal expiratory flow-pressure ($\dot{V}$max-P) curve. The slope of the $\dot{V}$max-P curve was used as an indicator of airway constriction. Dynamic respiratory compliance (Crs) was calculated as the ratio of the ventilated animal's tidal volume/ΔPao. The ΔPao was obtained as the difference between the Pao at the end of inspiration and the end of expiration.

Immediately following the functional tests, each anesthetized-ventilated animal's chest was opened. Pulmonary arterial pressure (Ppa) was measured by insertion of a 22 G needle into the pulmonary artery via the right ventricle and was recorded on the polygraph. The trachea (together with the main stem bronchi), lungs, right ventricle (RV) and left ventricle plus septum (LV+S) were then excised, dissected, and weighed. The weight ratio of the RV/(LV+S) was calculated. The trachea and left lung from each rat were then stored at -70°C for later analysis.

Neutral Endopeptidase (NEP) Assay

Tracheal NEP content was determined using a modification of the methods of Orlowski and Wilk

(23), Haxhiu-Poskurica et al. (9), and Kumar (personal communication). The frozen tracheas were thawed and minced into tubes containing 600 µl of 50 mM Tris, pH 7.4. The tissues were sonicated for 30 sec at 4°C and were centrifuged at 17500 g for 15 min. The supernatants were removed for NEP analysis. This extraction was repeated twice; the three supernatants from each rat were combined, frozen at -20°C, and assayed the next day.

The reaction mixture (250 µl) contained 50 µl of tissue extract, 1.25 mM (final concentration) glutaryl-alanine-alanine-phenylalanine-4-methoxy-2-naphthylamine substrate, 10 µg leucine aminopeptidase, and 50 mM Tris buffer, pH 7.4. The samples were incubated for 2 hours at 37°C. The reaction was stopped by addition of 1 ml of fast garnet GBC in 10% Tween 20 in 1 M sodium acetate, pH 4. 2, followed by 1 ml of water. The optical density was spectrophotometrically determined at 530 nm. The amount of 2-naphthylamine released was calculated from the extinction coefficient of the fast garnet GBC-2-naphthylamine reaction product (1). Each sample was run in duplicate. A 50 µl aliquot of each extract was also incubated for 15 min at room temperature with 8×10^{-7} M phosphoramidon in 50 mM Tris buffer, pH 7.4; substrate and leucine aminopeptidase were then added and the reaction was incubated as above. The protein concentration of the extracts was determined by the method of Lowry et al. (18). Phosphoramidon-inhibitable NEP specific activity was expressed as nmoles of 2-naphthylamine released per mg protein per hour.

Suhstance P (SP) and Neurokinin A (NKA) Assay

The lung levels of SP and NKA were assayed using an enzyme-immunoassay (EIA) procedure. The frozen lungs were thawed and minced into tubes containing 10 volumes of 2 N acetic acid (26). The samples were placed in a boiling water bath for 10 min, and were then sonicated to homogeneity. The extracts were centrifuged at 3500 g for 20 min. The supernatants were removed, aliquotted, lyophilized overnight, and stored at -70°C until the assays were performed.

On the day of the assay (for SP or NKA), one aliquot from each lung extract was reconstituted in half of the original volume with EIA buffer. The samples were mixed well and allowed to stand in an ice bath for 5 min. The buffer-insoluble components were removed by centrifugation at 3500 g for 5 min, and the supernatants were reserved for analysis.

Protein A-coated 8-well microwell strips were washed with 0.01 M KH_2PO_4/K_2HPO_4 buffer containing 0.05% Tween 20. Fifty µl of samples or SP (or NKA) standards, diluted anti-SP (or anti-NKA) antibody, and SP (or NKA) conjugated with horseradish peroxidase were added to the wells. Each sample was run in quadruplicate, and each 12-strip plate included two sets of standards. The plates were incubated for 2 hours with gentle shaking at room temperature. The wells were washed and 150 µl of K-Blue substrate (3, 3',5,5' tetramethylbenzidine with hydrogen peroxide, stabilized; Elisa Technologies, Lexington, KY) was added. The plates were incubated again with moderate shaking for 30 min (SP) or 2 hours (NKA). 1 00 µl of 1 N HCl was added to stop the reaction. The optical density at 450 nm was determined using a Biotech EL310 microplate reader (Biotech Instruments, Winooski, VT). The concentration of SP or NKA was determined by comparison to the standard curves, and was reported as pmoles per g wet lung.

Data Analysis

All values are expressed as means ± SEM. Overall comparisons were made using analysis of variance. Treatment groups were compared to the control group using Dunnett's test. Treatment groups were compared to each other using Scheffe's test. Differences were considered to be significant when $p < 0.05$. Regression analysis was used to compare the MCT+capsaicinl, MCT+capsaicin2, and MCT+ capsaicin3 groups (regression across time).

Results

Body Weight, Lung Weight, and Respiratory Parameters

The rats in the capsaicin+MCT group had significantly greater lung weight than the control rats (Table 1). All of the MCT+capsaicin groups had significantly lower body weight than either the control or capsaicin group animals; the MCT+capsaicin2 and the MCT+capsaicin3 group animals also had significantly lower body weight than the capsaicin+MCT rats. The MCT+capsaicin groups also had significantly greater lung weight than the control group. There were no significant differences in respiratory parameters between any of the three MCT+capsaicin groups and the control group. The rats in the MCT+capsaicin1 group had significantly lower maximal expiratory flow at 50% total lung capacity ($\dot{V}max_{50}$) than the rats in the capsaicin group (Table 1). No significant differences in lung weight or respiratory parameters were found between any of the MCT+capsaicin groups and the control or capsaicin+MCT groups.

Vascular Parameters

Capsaicin alone had no significant effect on

Table 1. Body Weigth, Lung Weight and Respiratory Parameters

Group	n	Body Weight (g)	Lung Weight (g)	TLC (ml)	FRC (ml)	V̇max50 (ml/sec)	Crs (ml/cm H_2O)	V̇max-P slope (ml/sec/cm H_2O)
Control	8	306±11	1.19±0.05	12.9±0.36	2.43±0.08	53.6±2.07	0.19±0.01	5.93±0.30
CAP	8	326±10	1.27±0.04	13.1±0.43	2.50±0.08	57.0±1.84	0.20±0.02	6.16±0.26
CAP+MCT	8	303±15	1.48±0.07[a]	11.3±0.39	2.13±0.13	49.5±1.42	0.17±0.01	5.82±0.38
MCT+CAP1	8	261±6	1.57±0.08[a]	11.8±0.33	2.29±0.07	47.8±2.07[b]	0.18±0.01	5.61±0.24
MCT+CAP2	7	243±8[a,b,c]	1.44±0.06[a]	11.4±0.48	2.51±0.22	49.8±1.14	0.17±0.02	5.72±0.39
MCT+CAP3	8	241±8[a,b,c]	1.47±0.06[a]	12.3±0.68	2.45±0.13	52.5±1.40	0.18±0.02	6.14±0.30

All values are mean ± SEM. CAP= capsaicin; MCT= monocrotaline. The control group received saline. CAP was injected before (CAP+MCT) or 7 (MCT+CAP1), 11 (MCT+CAP2), or 14 (MCT+CAP3) days after MCT. n, number or rats; TLC, total lung capacity; FRC, functional residual capacity; V̇amx50, maximal expiratory flow at 50%TLC; crs, dynamic respiratory compliance, V̇max-P slope, slope of maximal expiratory flow-static recoil pressure curve. Significant differences between groups ($p<0.05$): [a]compared to the control group; [b]compared to the CAP group; [c]compared to the CAP+MCT group.

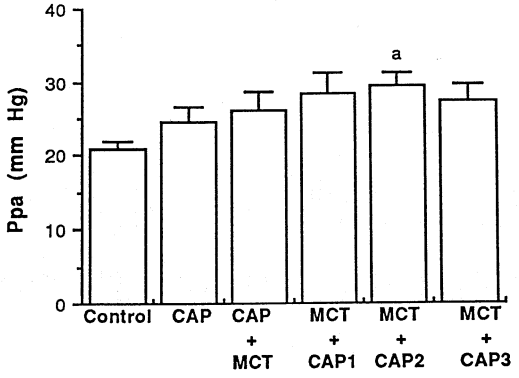

Fig. 1. Pulmonary arterial pressure (Ppa) in six groups of rats. CAP = capsaicin; MCT = monocrotaline. CAP was injected before (CAP+MCT) or 7 (MCT +CAPl), 11 (MCT +CAP2), or 14 (MCT +CAP3) days after MCT. Bars indicate SEM. Significant differences between groups ($p < 0.05$): [a]compared to the control group.

Table 2. Ventricular Weights

Group	n	RV (g)	LV+S (g)
Control	8	0.741±0.018	0.632±0.028
CAP	8	0.190±0.015	0.793±0.035[a]
CAP+MCT	8	0.199±0.020	0.672±0.027
MCT+CAP1	8	0.182±0.035	0.595±0.021[b]
MCT+CAP2	7	0.182±0.047	0.555±0.025[b]
MCT+CAP3	8	0.194±0.034	0.564±0.023[b]

All values are means ± SEM. The groups are the same as those in Table 1. n, number of ratss; RV, right ventricle weight; LV+S, left ventricle plus septum weight. Significant differences between groups ($p<0.05$): [a]compared to the control group; [b]compared to the CAP group.

Ppa. The rats in the MCT+capsaicin2 group had a significantly higher Ppa than the rats in the control group (Fig. 1).

Capsaicin alone significantly increased LV+S; the three MCT+capsaicin groups all had LV+S significantly lower than the capsaicin group (Table 2). Both the RV/BW and the (LV+S)/BW were significantly higher in the three MCT+capsaicin groups than in the control group (Fig. 2). The RV/BW was also significantly higher in the MCT+capsaicin2 and MCT+capsaicin3 groups than in the capsaicin group. The MCT+capsaicin2 and MCT+capsaicin3 groups showed RV/(LV+S) ratios significantly greater than that of the control group; the RV/(LV+S) was also significantly greater in the MCT+capsaicin3 group than was the ratio in the capsaicin group (Fig. 2).

Tachykinin Levels and NEP Activity

All capsaicin-treated animals had significantly lower lung SP levels than did control animals (Fig. 3). Capsaicin had no significant effect on NKA levels (Fig. 3), however. Capsaicin alone had no effect on the tracheal NEP level (Fig. 4). In adition, the NEP activity was not affected by MCT.

Discussion

We found previously that MCT increased lung SP levels and that capsaicin pretreatment significantly prevented MCT-induced both early airway dysfunction and late pulmonary hypertension (33). This study was carried out to elucidate the length of capsaicin treatment needed to prevent early or late MCT

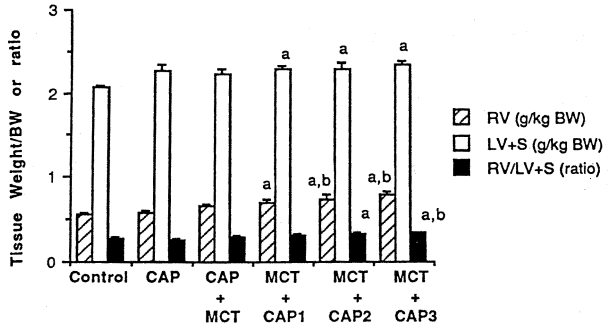

Fig. 2. Weight of right ventricle per kg of body weight (RV/BW), left ventricle plus septum per kg of body weight (LV+S/BW), and RV/LV+S ratio in six groups of rats. Groups are as described in Fig. 1. Bars indicate SEM. Significant differences between groups ($p < 0.05$): [a]compared to the control group; [b]compared to the CAP group.

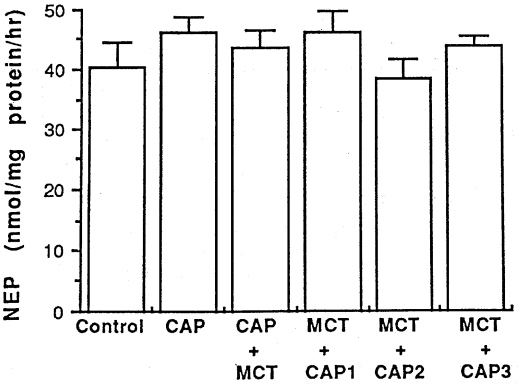

Fig. 4. Neutral endopeptidase (NEP) activities in six groups of rats. Groups are as described in Fig. 1. Bars indicate standard errors of the mean.

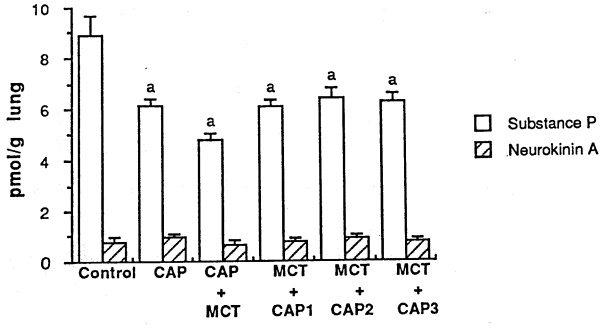

Fig. 3. Tachykinin levels in six groups of rats. Groups are as described in Fig. 1. Bars indicate standard errors of the mean. Significant differences between groups ($p < 0.05$): [a]compared to the control group.

pneumotoxicity. The working hypothesis of this study was that chronic capsaicin treatment would block the pneumotoxic effects of MCT in a time-dependent manner. Capsaicin administration 1 week after MCT injection was expected to block the development of any actions of MCT that required the presence of increased tachykinin levels for more than 7 days, but tachykinin-mediated effects occurring in less than 1 week would remain unaffected. Capsaicin administration 11 or 14 days after MCT injection was expected to prevent or attenuate only those effects of MCT that required increased tachykinin levels for 1.5-2 weeks for development or progression of the effect.

In this current study, MCT in the presence of capsaicin (pretreatment or posttreatment) had no significant effect on lung volumes, $\dot{V}max_{50}$, or $\dot{V}max$-P slope, although these measures were generally lower in rats that received both MCT and capsaicin than in the non-MCT-treated rats. It is difficult to determine if these results were due to antagonism of MCT by capsaicin; the apparent blockade may merely reflect the inconsistency of MCT-induced effects on flow and volume. Lai et al. (16) found that FRC and TLC were decreased in young adult (approximately 300 g) rats 1 and 2 weeks after MCT administration (60 mg/kg), but the rats in the 3 week post-MCT test group showed a smaller, nonsignificant decrease. Zhou & Lai (32) found that 60 mg/kg of MCT reduced TLC, VC, and FRC 1 week after administration to 240-g rats. Gillespie et al. (7) reported that 105 mg/kg of MCT decreased TLC in much younger (100-125 g) rats. It has been suggested that tachykinins mediate the progression of ventilatory dysfunction (33); the 60 mg/kg dose of MCT used in this study may represent a threshold of activity for volume and flow alterations in rats of this size, age, or strain.

Monocrotaline-induced decreases in Crs and $\dot{V}max$-P slope could be prevented or attenuated by capsaicin pretreatment (33). The results of the present study show that capsaicin pretreatment as well as capsaicin posttreatment at any time from 1 to 2 weeks after MCT administration blocked the previously observed decreases in Crs and $\dot{V}max$-P slope. These data suggest that increased airway constriction and lung stiffness are reversible, tachykinin-mediated effects, dependent on the continued presence of increased tachykinin levels. Capsaicin administration 1-2 weeks after MCT injection would deplete tachykinins, resulting in reversal of the MCT-induced changes. It is also possible that the MCT-induced changes in Crs and $\dot{V}max$-P slope are tachykinin-dependent but rapid and transient; this could account for the apparent lack of temporal dependence of the blockade. However, Zhou and Lai (33) demonstrated that MCT significantly decreased all of these measures both 1 and 3 weeks after administration. Therefore, the increases in lung stiffness and airway constriction are probably early (observable 1 week after MCT injection), reversible, tachykinin-mediated effects.

Pulmonary arterial pressure was significantly increased only in the MCT+capsaicin2 group. It has been shown that significant MCT-induced pulmonary hypertension appears from 12 days (25) to 3 weeks (10, 21, 22, 32) after injection; the MCT metabolite monocrotaline pyrrole has been reported to cause a significant increase in Ppa as soon as 7 days after administration (4). Capsaicin pretreatment has previously been shown to block the MCT-induced increase in Ppa (33). The results of the present study show a small but nonsignificant increase in Ppa in the capsaicin-only and capsaicin pretreatment groups; therefore, prolonged depletion of tachykinins had a protective effect. The failure of capsaicin to block the MCT-induced increase in Ppa in one of the late depletion groups indicates that the presence of elevated tachykinins for an extended period of time was required for the development of pulmonary hypertension. It is difficult to discern the precise time course of development of pulmonary hypertension in this experiment, however, due to the similarity between the three capsaicin posttreatment groups. Nonetheless, these data suggest that pulmonary hypertension is at least partially tachykinin-dependent, and the tachykinin-mediated component of action requires the presence of increased tachykinins for more than one week.

Three weeks after MCT administration, the increase in the ratio RV/(LV+S) in this study was mainly due to the increase in RV. This effect was blocked by early (capsaicin pretreatment and 1 week posttreatment), but not late (capsaicin treatment 11 or 14 days post MCT), depletion of tachykinins, suggesting that right ventricular hypertrophy is also at least partially tachykinin-dependent and, as in pulmonary hypertension, elevated tachykinins must be present for more than one week. Several other agents, not usually associated with tachykinin activity, have been shown to reduce the right ventricular hypertrophy and pulmonary hypertension produced by MCT. Two platelet-activating factor antagonists, WEB 2086 and WEB 2170, partially blocked the elevation of Ppa by MCT (22). The polyamine biosynthesis inhibitor, α-difluoromethylornithine, prevented the elevation of Ppa 3 weeks after MCT (21); this in turn prevented increased DNA synthesis (8). Mild hyperoxia reduced the late-developing MCT-induced hypertensive effects as well as the increase in pulmonary artery medial thickness (11). Hypoxia results in increased SP release in the brain (17) and increased SP levels in the lung (14), but it is not known if the protective effect of hyperoxia implies that MCT mimics hypoxic effect in the lung. The relationship between these agents is not yet clear, but it is likely that more than one basic mechanism mediates their attenuation of MCT pneumotoxicity. Capsaicin administration was expected to deplete tachykinins (5, 6). In this report, we found that SP levels in capsaicin-treated rats were decreased an average of 33% with respect to saline-treated control rats. The capsaicin+MCT group had a significantly lower level of SP than that found in the capsaicin-posttreatment groups (considered as a whole). It has been demonstrated that MCT acts to increase the synthesis of SP (33); since capsaicin administration results in degeneration of afferent C fibers (12), pretreatment with capsaicin could prevent MCT from stimulating SP biosynthesis. Capsaicin posttreatment, on the other hand, would still decrease the level of SP, but that level would be slightly higher due to MCT-induced SP biosynthesis or transport. Although capsaicin administration was expected to decrease NKA levels (29), no reduction was seen. This may be an artifact due to the large variability seen between the rats. Also, the EIA technique used may not have been sensitive enough to detect changes in such low NKA values. NKA has been reported to be a more potent bronchoconstrictor in guinea pig trachea than SP, although NKA was present in much lower concentrations (30).

There was no decrease in the activity of NEP as a result of either capsaicin or capsaicin+MCT administration. Since the tissues were removed 3 weeks after MCT administration, it is possible that NEP activity was only suppressed for a short time, and had returned to normal within 3 weeks. This was not found in the study of Zhou & Lai (33), but that study only extended NEP analysis to 2 weeks after MCT. The tracheal activity of NEP observed in this study (40-50 nmol/mg protein/hr) was substantially higher than that previously found in rats (24-50 pmol/mg protein/hr) (3, 33) and young piglets (approximately 0.6 nmol/mg protein/hr) (9), although even higher activities were reported in guinea pig tracheas (about 2400 nmol/mg protein/hr) (19). There are three main reasons for these discrepancies. First, most of the reports cited above did not use the substrate that was used in this study. Second, the reactions were not stopped with trichloroacetic acid (TCA) in this experiment, since TCA rapidly degraded the 2-naphtylamine-fast garnet reaction product (unpublished observation). Third, 2-naphthylamine is highly toxic; we therefore calculated the concentration of the reaction product using the molar extinction coefficient of 27,000 published by Barrett (1) rather than comparing the results to a standard curve.

It appears that the early (developing within 1 week) and late (developing after 2-3 weeks) effects of MCT have different primary mechanisms. Moreover, some early effects (such as the decreases in BW, Crs, and $\dot{V}$max-P slope) may be primarily tachykinin-

mediated while other early effects such as lung edema may be mediated mainly by polyainines (20). It has been suggested that the later effects of MCT (pulmonary hypertension and right ventricular hypertrophy) are dependent on the occurrence of the early ventilatory dysfunction (16). Although the results of the present study are consistent with this hypothesis, the ability of verapamil to prevent the early MCT-induced vascular leak without preventing subsequent pulmonary hypertension (27) implies that the later effects may not be entirely dependent on the occurrence of all of the earlier effects. The experiments conducted by Hill et al. (11) led them to conclude that hypoxia contributed to MCT-induced pulmonary hypertension but not to earlier effects; this could also indicate a difference in the mechanisms of the early and late effects.

In summary. it appears that MCT acts in a biphasic manner. The early effects of MCT probably reflect both tachykinin-mediated (BW and airway dysfunction) and non-tachykinin-mediated (possibly lung edema) mechanisms of action. The early tachykinin-mediated effects do not appear to require a prolonged increase in tachykinin levels. The mechanisms of action of the early non-tachykinin-mediated effects of MCT may include increased polyamine synthesis (20). The later effects of MCT (pulmonary hypertension and right ventricular hypertrophy) may also have both tachykinin-mediated and tachykinin-independent components of action. In contrast to the early tachykinin-mediated component of action, the late tachykinin-mediated component probably requires the presence of elevated tachykinin levels for a prolonged period of time.

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