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Possible Link Between Brain Serotonin Metabolism and Methionine Sulfoximine-Induced Hypothermia and Associated Behavior in the Rat

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GINEFRI-GAYET, M. AND J. GAYET. *Possible link between brain serotonin metabolism and methionine sulfoximine-induced hypothermia and associated behavior in the rat.* PHARMACOL BIOCHEM BEHAV **43**(1) 173–179, 1992.—L-Methionine-D,L-sulfoximine (MSO) intraperitoneally or intracerebroventricularly (third ventricle) injected at convulsant doses induced a hypothermia, primarily associated with a syndrome of ataxia, in the restrained rat maintained at an ambient temperature of 23°C. Depletion of brain serotonin (5-HT) by pretreatment with *p*-chlorophenylalanine (PCPA), *p*-chloroamphetamine (PCA), and *d*-fenfluramine (FFA) did not significantly modify the time course and magnitude of MSO-induced developing hypothermia but it enhanced abnormal motor behavior. Enhancement of 5-HT synthesis in MSO-submitted rats pretreated with 5-hydroxytryptophan (5-HTP) (200 mg/kg, IP) alone or 5-HTP (100 mg/kg, IP) preassociated with carbidopa (10 mg/kg, IP) suppressed significantly hypothermia, but it did not greatly modify motor disturbances. In conclusion, the neurocytochemical processes initiating hypothermia following administration of MSO to the rat appear to be linked to a slowdown of the rate of brain 5-HT turnover, maybe at the level of the midbrain raphe nuclei.

Methionine sulfoximine Hypothermia Motor behavior Serotonin metabolism
p-Chlorophenylalanine *p*-Chloroamphetamine *d*-Fenfluramine 5-Hydroxytryptophan

THE convulsant molecule L-methionine-D,L-sulfoximine (MSO) intraperitoneally or intracerebroventricularly administered induced a decrease of body temperature in the restrained rat kept at an ambient temperature of 23°C; this hypothermia appeared to be a poikilothermia-like state in the cold environment with retention of a normal regulation in the warm environment (19). It has been suggested that this effect of MSO on body temperature was mediated within the CNS and that it might be directly related to a depressive action on glucose oxidative metabolism in cerebral cell structures, maybe astroglial cells, probably located in the vicinity of the ventricle or capillary walls (19). The MSO-elicited hypothermia developed during the 3- to 6-h latency period preceding that of an episodic running behavior and/or generalized seizures (19,56). Probably, this relatively long preconvulsive period would be associated with the building up of the neurocytochemical processes, involving potentially some particular neurotransmitters or neuromodulateurs, related to or directly responsible for the subsequent seizures. A study was previously made on the effects of MSO on the regional rate of serotonin (5-HT) turnover in the rat brain: The findings reflected a complex, temporal pattern of regional MSO action (50). Over a 10- to 40-min experimental period, the findings suggested a slowdown of the 5-HT turnover in the brainstem, midbrain, and striatum, but not the cerebral cortex; over a 5-h experimental period, MSO effect suggested a decreased rate of 5-HT turnover in the cerebral cortex, brainstem, and striatum, and over a 12-h experimental period MSO effect suggested an increased rate of 5-HT turnover in the midbrain and hypothalamus (50). In C57BL/6J mice pretreated with a convulsant dose of MSO, brain 5-HT and plasma and brain tryptophan concentrations decreased 4 and 8 h after administration of the drug, and brain 5-hydroxyindoleacetic acid (5-HIAA) levels were not significantly altered, suggesting an impairment in 5-HT synthesis resulting from a restriction of tryptophan availability (8). As pointed out, the approximately 20% decrease in whole-brain 5-HT seen 8 h after the convulsant dose was small in magnitude but if selectively applied to a functionally relevant pool could have a major impact on the effects of 5-HT neurons (8). Recent experiments confirmed that MSO, at a convulsant

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dose, induced a mean 50% decrease of 5-HT concentration in seven areas of the rat brain 8 h after administration, that is, during the period of episodic seizures; in the same experimental conditions, there were no obvious changes in tryptophan and 5-HIAA levels in any area examined (20). Treatment of rats with a high dose of 5-hydroxytryptophan (5-HTP), the immediate precursor of 5-HT, alone or jointly with an inhibitor of peripheral 5-HTP decarboxylase, retarded MSO-induced seizure onset and significantly prevented seizures, as well as decrease in brain 5-HT content (20,50). In considering all these data, it appears that MSO must exert its action on the rate of brain 5-HT synthesis.

The progressive and relatively long-lasting hypothermia following systemic administration of a convulsant dose of MSO to the rat may be related to an impairment of 5-HT metabolism in the CNS because evidence was provided favoring a pivotal role for this indoleamine in the diencephalon's control system for body temperature (30,35,40).

The present experiments were designed to determine if a relationship could exist between the slowdown of the rate of 5-HT turnover in the CNS and the decrease of body temperature in the rat submitted to the action of a convulsant dose of MSO, the molecule being systemically and intracerebroventricularly administered. The effects of a reduction in the level of 5-HT were studied either by an acute pretreatment with a high dose of *p*-chlorophenylalanine (PCPA), an inhibitor of the rate-limiting step in the 5-HT synthesis (3,10,24,28,31), or pretreatment with the two amphetamine derivatives *p*-chloroamphetamine (PCA) and *d*-fenfluramine (FFA), which induced release of 5-HT followed by persistent decreases in forebrain levels of 5-HT chemical markers, reflecting degeneration of 5-HT axon terminals in many regions of the forebrain (16,34,38,44,47). Conversely, a 5-HTP loading was performed by administration of the immediate precursor of 5-HT alone or jointly by pretreatment with carbidopa, an inhibitor of peripheral 5-HTP decarboxylase, inducing an increase of 5-HT content in the CNS (4,6,9,32,33,46,54).

METHOD

Animals

Male Wistar (Allingthon Farms) rats were purchased from the breeder (CERJ, Le Genest-St. Isle, France). Animals weighing between 225–325 g were used throughout the experiments; they were housed individually in Plexiglas cages in a temperature-controlled environment of 23 ± 1.0°C with a 12 L : 12 D cycle (lights on 0700–1900 h) and with free access to food (Extralabo, Ste. Colombe, M25.C diet) and water. All experiments began between 0900 and 1100 h.

Measurement of Body Temperature

The rat was placed in a well-ventilated Harvard universal acrylic restrainer. Rectal temperature was monitored using a lubricated temperature probe (Yellow Springs Instruments Co., Yellow Springs, CO, model 402) inserted 6 cm into the rectum, with a telethermometer (Yellow Springs Instruments, model 43) connected to a potentiometric recorder (Sefram, Paris, Servotrace model PE-10). The probe was secured in place by taping it lightly to the base of the tail. A control measurement (0 time) was taken immediately after administration of MSO and rectal temperature was recorded continuously thereafter from 30 min–5 h later.

IP Injection of MSO

MSO (Sigma Chemical Co., St. Louis, MO) (100–200 mg/kg body weight), dissolved in 1.0 ml sterile 0.9% NaCl solution per 200 g body weight, was injected intraperitoneally; control animals received the same volume of sterile 0.9% NaCl solution.

ICV Injection of MSO

One week prior to the experiment, the rat was placed in a David Kopf model 900 stereotaxic apparatus, under equithesin (3 ml/kg, IP) anesthesia. A 26-ga guide cannula cut to a length of 13.0 mm from stainless steel tubing (Hamilton) was implanted perpendicularly to the surface of the skull, with the tip resting 1.0 mm above the third ventricle. The coordinates (2) were (mm): A = 6.1, L = 0.0, H = 3.5; the cannula was held in place by dental cement and stainless steel anchor screws inserted into the calvarium (39). A stainless steel stylet cut to a length of 13.0 mm was placed into the guide cannula and left until the experiment began.

MSO (50–75 μg per rat), dissolved in sterile 0.9% NaCl solution or artificial cerebrospinal fluid (41), was injected in a volume of 10 μl over a period of 2.30 min through a 33-ga injector needle cut to a length of 14.0 mm from stainless steel tubing (Hamilton), connected with a length of polyethylene tubing (internal diameter 0.30 mm) to a 50-μl capacity Hamilton microsyringe driven by a variable-speed infusion pump (Braun, Melsungen, model Perfusor I), as previously described (19).

At the conclusion of each experiment, the location of the site of injection was verified with the method of Myers (39), using a 1% bromophenol blue solution in bidistilled water; those preparations in which the dye was visible in all the cerebroventricular cavities were regarded as valid.

Behavioral Observations

At the end of the 5-h record of rectal temperature, the rat was immediately and carefully taken out of the restrainer, placed in a Plexiglas box, and its behavior observed for 15 min in the same environmental conditions.

Drug Treatment

PCPA HCl (Sigma), dissolved in 0.9% NaCl solution, was intraperitoneally injected at a dose of 300 mg/kg and in a volume of 1.0 ml/200 g body weight 48 h before administration of MSO or 0.9% NaCl solution for control rats.

The procedure described by Mamounas and Molliver (34) was used for pretreatment with PCA HCl (Sigma). Immediately prior to PCA treatment, animals were placed in a cold (12 ± 1.0°C), ventilated, and lighted box; they were housed one rat per Plexiglas cage. Rats were administered two intraperitoneal injections (24 h apart; 1100 h each day) of PCA (6 mg/kg, dissolved in 0.9% NaCl solution and injected in a volume of 1.0 ml/200 g body weight). Twenty-four hours after the second injection, animals were replaced in the normal ambient temperature of 23°C and MSO or 0.9% NaCl solution for control rats administered 5 days later.

FFA HCl (Institut de Recherches Internationales Servier, Neuilly), dissolved in 0.9% NaCl solution, was subcutaneously injected at a dose of 40 mg/kg and in a volume of 1.0 ml/200 g body weight 24 h before administration of MSO or 0.9% NaCl solution for control rats (27).

5-HTP (Sigma), freshly dissolved in warmed 0.9% NaCl solution, was intraperitoneally injected at doses of 50–200

TABLE 1

CHANGE IN RECTAL TEMPERATURE PRODUCED BY AN INTRAPERITONEAL INJECTION TO RESTRAINED RATS OF MSO AFTER PRETREATMENT WITH 5-HT DEPLETORS (PCPA, PCA, AND FFA) AND AFTER PRETREATMENT WITH 5-HTP (ALONE OR TOGETHER WITH CARBIDOPA)

Pretreatment	MSO (mg/kg)	Change in Rectal Temperature	Number of Rats per Group
Saline	100	−2.8 ± 0.4*	4
Saline	150	−2.2 ± 0.3*	7
Saline	200	−2.4 ± 0.2†	4
PCPA (300 mg/kg, IP)	150	−3.2 ± 0.3*	4
PCA (2 × 6 mg/kg, IP)	150	−2.3 ± 0.3*	4
FFA (40 mg/kg, SC)	150	−2.9 ± 0.3*	4
5-HTP (100 mg/kg, IP)	150	−2.6 ± 0.4*	5
5-HTP (200 mg/kg, IP)	150	−0.4 ± 0.2	4
Carbidopa (10 mg/kg, IP) + 5-HTP (50 mg/kg, IP)	150	−1.3 ± 0.1†	4
Carbidopa (10 mg/kg, IP) + 5-HTP (100 mg/kg, IP)	150	−0.7 ± 0.3‡	4

Values are mean maximum change in rectal temperature ± SEM compared with corresponding saline controls for each group of rats. ‡$p < 0.05$, *$p < 0.01$, and †$p < 0.001$, for significant differences compared with corresponding saline controls (evaluated by Student's *t*-test).

mg/kg and in a volume of 1.0 ml/200 g body weight 15 min before administration of MSO or 0.9% NaCl solution for control rats. Carbidopa (Merck Sharp and Dohme, Rahway, NJ), dissolved by means of equimolar amount of HCl diluted in 0.9% NaCl solution, was intraperitoneally injected at a dose of 10 mg/kg and in a volume of 0.5 ml/200 g body weight 30 min prior to administration of 5-HTP.

RESULTS

Mean rectal temperature of normal restrained rats was 38.0 ± 0.4°C at an ambient temperature of 23 ± 1.0°C and in the light phase; an intraperitoneal injection of 0.9% NaCl solution (1.0 ml/200 g body weight) or an injection of artificial cerebrospinal fluid into the third ventricle (10μl per rat) did not exert any significant effect on rectal temperature. An intraperitoneal dose of 100–200 mg/kg MSO or an intracerebroventricular dose of 60 μg per rat of the drug induced a significant decrease in rectal temperature of 2.2 ± 0.3 to 2.8 ± 0.4°C or 2.6 ± 0.3°C, respectively (Tables 1 and 2). The nadir of the decrease in rectal temperature was reached during the course of the 0400–0500 h period or of the 0200–0230 h period following MSO administration, respectively. This hypothermia was associated with the development of a marked syndrome of ataxia with the loss of righting reflexes, an impairment of postural adjustment, and hindlimb abduction.

Effects of Depletion of 5-HT on MSO-Induced Hypothermia and Associated Behavior

As stated in the introductory paragraph, MSO-submitted rats were pretreated with each of the three 5-HT-depleting molecules, namely PCPA, PCA, and FFA.

Pretreatment with a unique high dose of PCPA (300 mg/kg, IP) was followed by a slight, nonsignificant ($p > 0.05$) increase of MSO-induced hypothermia (Fig. 1A; Tables 1 and 2). Rats exhibited a syndrome of ataxia associated with hindlimb extension and abduction and eventually with tonic-clonic generalized seizures.

Results obtained with MSO-submitted rats pretreated either with PCA (two intraperitoneal injections of 6 mg/kg) or FFA (40 mg/kg, SC) resembled those obtained with PCPA-pretreated animals. MSO-induced decrease of rectal temperature was not statistically different from that recorded in rats

TABLE 2

CHANGE IN RECTAL TEMPERATURE PRODUCED BY AN INJECTION OF MSO INTO THE THIRD VENTRICLE OF RESTRAINED RATS AFTER PRETREATMENT WITH 5-HT DEPLETORS (PCPA AND FFA) AND AFTER PRETREATMENT WITH 5-HTP

Pretreatment	MSO (μg/rat)	Change in Rectal Temperature	Number of Rats per Group
Saline	60	−2.6 ± 0.3*	5
PCPA (300 mg/kg, IP)	60	−2.4 ± 0.2†	3
FFA (40 mg/kg, SC)	60	−2.3 ± 0.1†	4
5-HTP (200 mg/kg, IP)	60	+0.8 ± 0.1*	5

Values are mean maximum change in rectal temperature ± SEM compared with corresponding saline controls for each group of rats. †$p < 0.01$ and *$p < 0.001$, for significant differences compared with corresponding saline controls (evaluated by Student's *t*-test).

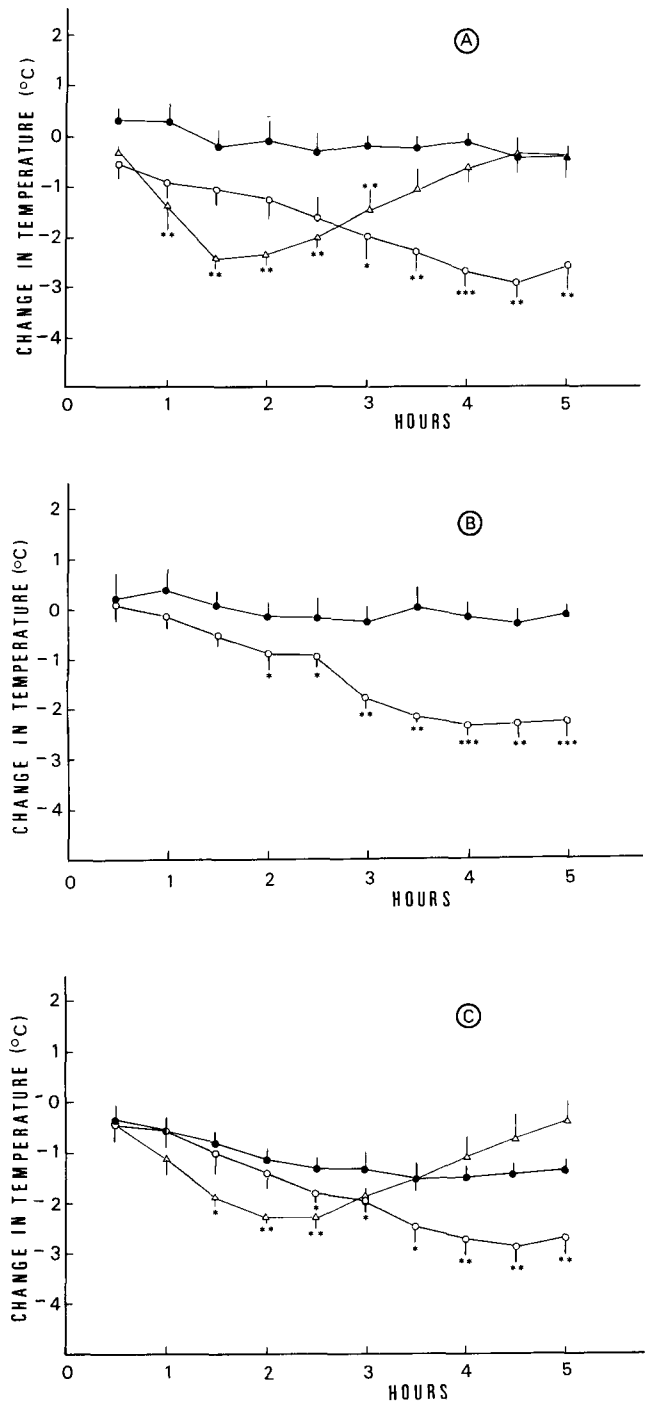

FIG. 1. Change in rectal temperature following administration of MSO to restrained rats at an ambient temperature of 23 ± 1.0°C. (A) Pretreatment with PCPA (300 mg/kg, IP). (○), MSO (150 mg/kg, IP) (n = 4); (△), MSO (60 μg per rat in the third ventricle) (n = 3); (●), saline controls (n = 4). (B) Pretreatment with PCA (2 × 6 mg/kg, IP). (○), MSO (150 mg/kg, IP) (n = 4); (●), saline controls (n = 4). (C) Pretreatment with FFA (40 mg/kg, SC). (○), MSO (150 mg/kg, IP) (n = 4); (△), MSO (60 μg per rat in the third ventricle) (n = 4); (●), saline controls (n = 4). Time in hours. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, for significant differences compared with saline controls (evaluated by Student's t-test).

receiving MSO alone (Figs. 1B and 1C; Tables 1 and 2). Animals thus pretreated with PCA and FFA, then observed unrestrained 5 h after administration of MSO, exhibited a syndrome of ataxia associated with a flat body posture and an early starting of tonic-clonic generalized seizures, particularly in FFA-pretreated rats then submitted to an injection of MSO into the third ventricle.

Rectal temperature of control rats pretreated with PCPA, PCA, or FFA, recorded for 5 h, did not significantly vary (Fig. 1) and behavior of all animals appeared normal.

Effects of 5-HTP Loading on MSO-Induced Hypothermia and Associated Behavior

MSO-submitted rats were pretreated with an IP injection of 5-HTP alone. At a dose of 100 mg/kg 5-HTP, the decrease of rectal temperature induced by an IP injection of MSO did not significantly differ from that recorded in rats receiving MSO alone (Fig. 2; Table 1), but all animals exhibited some inertia without muscle hypertonia or clonus. Pretreatment with 5-HTP at a dose of 200 mg/kg (IP) suppressed MSO-induced hypothermia and even a nonsignificant transient slight hyperthermia developed (Fig. 2; Tables 1 and 2); rats showed a syndrome of inertia, with blockade of righting reflexes associated with abnormal hindlimb extension and flexion but without any manifestation of muscle hypertonia. Rats receiving MSO into the third ventricle exhibited a marked flat body posture. Control animals pretreated with 5-HTP (100 and 200 mg/kg, IP) alone showed a nonsignificant decrease of body temperature (Fig. 2) and their motor behavior was apparently normal.

In a second series of experiments, MSO-submitted rats were pretreated with 5-HTP preassociated with carbidopa. In rats pretreated with 5-HTP (50 mg/kg, IP) preassociated with carbidopa (10 mg/kg, IP) and subsequently submitted to MSO (150 mg/kg, IP), recorded rectal hypothermia was approximately 50% lower than that recorded in control rats (Fig. 3A; Table 1); in these experimental conditions, all animals exhibited some inertia together with a flat body posture. Pre-

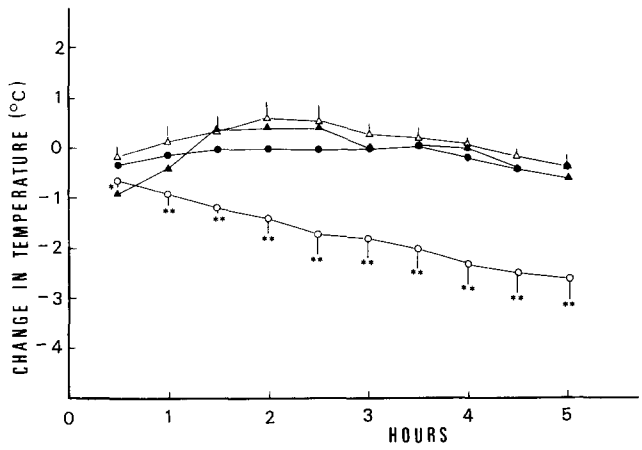

FIG. 2. Change in rectal temperature following administration of MSO to restrained rats at an ambient temperature of 23 ± 1.0°C. Pretreatment with 5-HTP (100 mg/kg, IP): (○), MSO (150 mg/kg, IP) (n = 4); (●), saline controls (n = 4). Pretreatment with 5-HTP (200 mg/kg, IP) ; (△), MSO (150 mg/kg, IP) (n = 4); (▲), saline controls (n = 4). Time in hours. SEM for saline controls are not represented. * $p < 0.05$ and ** $p < 0.01$, for significant differences compared with saline controls (evaluated by Student's t-test).

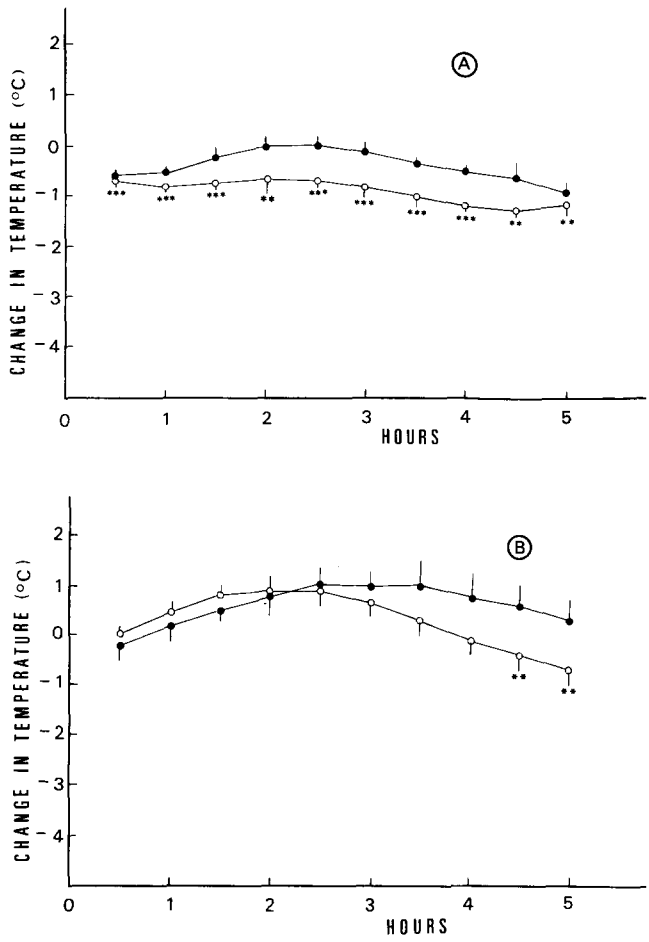

FIG. 3. Change in rectal temperature following administration of MSO to restrained rats at an ambient temperature of 23 ± 1.0°C. (A) Pretreatment with 5-HTP (50 mg/kg, IP) preassociated with carbidopa (10 mg/kg, IP). (○), MSO (150 mg/kg, IP) (n = 4); (●), saline controls (n = 4). (B) Pretreatment with 5-HTP (100 mg/kg, IP) preassociated with carbidopa (10 mg/kg, IP). (○), MSO (150 mg/kg, IP) (n = 4); (●), saline controls (n = 4). Time in hours. $^{**}p < 0.01$ and $^{***}p < 0.001$, for significant differences compared with saline controls (evaluated by Student's t-test).

treatment with 5-HTP (100 mg/kg, IP) preassociated with carbidopa (10 mg/kg, IP) not only suppressed hypothermia elicited by an IP injection of MSO but also caused a nonsignificant, transient, slight hyperthermia (Fig. 3B; Table 1). The rectal temperature of control rats was not significantly different from that of MSO-submitted rats except during the course of the 0400–0500 h period (Fig. 3B) and their motor behavior appeared normal.

DISCUSSION

MSO administered at a convulsant dose elicited a time-dependent regional perturbation of 5-HT metabolism in the rodent brain (8,20,50). The effect of MSO on the biosynthesis of brain [^{14}C]5-HT from intraarterially administered [^{14}C]5-HTP was previously examined in rats pretreated with the peripheral 5-HTP decarboxylase inhibitor Ro 4-4602: The findings led to the experimental evidence supporting a relatively early, although transient, slowdown of 5-HT turnover in the cell-body rich brainstem and midbrain regions (50). Thus, throughout the preconvulsant 5-h period, and as early as the first 10–40 min, MSO would decrease the rate of 5-HT turnover in perikarya located in the brainstem and midbrain and later this effect would propagate to the 5-HT axon terminals, especially in cerebral cortex and hippocampus (50). The perikarya of most 5-HT neurons are located within the midline raphe nuclei of the brainstem and project directly to most areas of the brain and spinal cord; ascending projections primarily originate in two major nuclei: the dorsal raphe nucleus (DRN) and the median raphe nucleus (MRN) (52).

At the present time, no explanation has been provided concerning the neurocytochemical mechanism(s) of the effect of MSO on the 5-HT neuron system during the long-lasting preconvulsant period. It has been demonstrated that MSO had no effect on the activity of 5-HTP decarboxylase (50). The irreversible inhibition of the activity of glutamine synthetase by MSO (36) led to a marked rise of ammonia levels in extra- and intracellular compartments of the rodent brain: In the arterial blood and cerebrospinal fluid, the values approached 0.3 mM/kg wet weight and in the brain it was close to 1.0 mM/kg wet weight at 0130 h (12,15,22). It was found that a concentration of ammonia from 0.1–10 mM stimulated the secretion of amines, in particular 5-HT, from synaptosomes isolated from rat brain in a dose-dependent manner: Ammonia induced an alkalinization of the intrasynaptic storage vesicles, which caused an extrusion of amines into the cytoplasm and their subsequent leakage into the medium through reversal of the plasma membrane transporters (14). Thus, the progressive increase of ammonia content in the brain, up to 4–5 h following systemic injection of MSO, would be quantitatively adequate to induce a leakage of indoleamine from 5-HT neurons, maybe facilitated by increased fluidity of the plasma membrane due to MSO-induced increased synthesis of phosphatidyl-*N*-monomethylethanolamine (49,51). The early effect of MSO on 5-HT metabolism in the perikarya-rich brainstem region might be explained by the greater vulnerability of this brain region to elevated ammonia levels (13,45).

During the first series of our experiments, the effects of a reduction in brain 5-HT levels were studied by acute pretreatment with PCPA, PCA, and FFA. PCPA caused an inhibition of 5-HT synthesis, mostly in the forebrain but less in the raphe nuclei (1), together with a peripheral and central 5-HT depletion (3,11,28,31). PCA and FFA induced 5-HT release from the synaptic extravesicular compartment (17,18,29,48,55) linked in the forebrain to a selective loss of fine (from DRN) but not beaded (from MRN) 5-HT axon terminals (7,34,38), thus leading to long-lasting reductions in cerebral 5-HT levels (16,26,44,47,53). There appeared differences in the rate of 5-HT depletion between discrete regions in the rat brain following treatment with PCPA, PCA, and FFA (37,53), indicating that they had a restricted neurotoxic effect upon specific structural components of 5-HT neurons: PCPA-induced depletion of 5-HT in the midbrain raphe nuclei was only about 50% whereas in the forebrain, which contains only fibers and terminals, the depletion was greater than 80% (1); on the other hand, cell bodies in the DRN and MRN were spared after PCA and FFA administration (34,38). We have found that pretreatment of MSO-submitted rats with these 5-HT depletors either lightly potentiated or did not alter hypothermia; however, the resulting motor troubles strengthened.

If the central 5-HT system would control or modulate MSO-induced hypothermia, it appears necessary to examine the effects of 5-HTP loading. As previously found (32), treatment of rats with 5-HTP (100 mg/kg, IP) alone resulted in an initial increase in the rate of brain 5-HT turnover, compared with concurrent controls, for the first 30 min, immediately

followed by its rapid decline up to its suppression at 6 h. In these experimental conditions, the time course and magnitude of MSO-elicited hypothermia were not modified. Pretreatment of MSO-submitted rats with a higher dose of 5-HTP (200 mg/kg, IP) alone not only suppressed hypothermia but also rapidly induced either normothermia (following injection of MSO into the third ventricle) or mild hyperthermia (following IP injection of MSO), although control rats developed a mild hypothermia. It has been found that the turnover of cerebral 5-HT appeared to increase as a function of the dose of 5-HTP injected and may be associated with an anomalous route of brain 5-HT metabolism (57). Pretreatment with 5-HTP preassociated with carbidopa attenuated by about 50% (5-HTP at a dose of 50 mg/kg, IP) or suppressed (5-HTP at a dose of 100 mg/kg, IP) MSO-induced hypothermia. Treatment of rats with 5-HTP (100 mg/kg, IP) preassociated with carbidopa (10 mg/kg, IP) was followed by an increase in the rate of brain 5-HT turnover that persisted for 6 h: The increases in 5-HT synthesis at 2, 4, and 6 h greatly exceeded those observed without carbidopa (32). It has been suggested that carbidopa exerted its predominant effect during distribution of 5-HTP; increased distribution of 5-HTP into the brain following pretreatment with carbidopa was not only the consequence of peripheral 5-HTP decarboxylase inhibition (43,57) but also resulted from inhibition of 5-HTP decarboxylase in the cerebrovascular endothelium (5) and even partly in the brain parenchyma (32). However, 5-HTP loading would be topographically and/or functionally distinct from endogenous 5-HT in the absence of exogenous precursor (25). Furthermore, some pharmacological effects of 5-HTP reflect an interference with central catecholaminergic transmission: A portion of exogenously administered 5-HTP may enter cerebral catecholamine-containing neuronal terminals, undergo decarboxylation to 5-HT, and then displace the endogenous catecholamine from vesicular stores (42). In conclusion, it appears that an increase in the rate of brain 5-HT turnover may interfere with the neurocytochemical processes of hypothermia following administration of MSO.

As recently stated (21), systemic administration of drugs acting on the 5-HT system does not provide information on the specific brain regions involved in 5-HT-dependent functions like thermoregulation in the rat; furthermore, peripheral factors may be a contributing element (23). Injection of MSO into the third ventricle, allowing the drug to interact more directly not only with thermoregulatory centers in the hypothalamus but also with brainstem and midbrain neuronal structures, led to a rapid decrease of body temperature, reaching its maximum value during the course of the 0200–0230 h period following administration of MSO. Pretreatment of rats with PCPA and FFA modified neither the time course nor the magnitude of the developing MSO-elicited hypothermia in these experimental conditions. We may put forward the following hypothesis: Extracellular 5-HT secreted from neuronal structures located in the brainstem and midbrain regions following elevation of ammonia levels, due to inhibition of glutamine synthetase by MSO, as explained above, would rapidly link to some 5-HT_1 receptor subtype(s) located in some 5-HT perikarya and dendrites in the brainstem and midbrain, maybe the DRN, eliciting a decrease of body temperature. As previously stated, a consequence of increased concentrations of 5-HT in the synaptic cleft is a reduced rate of synthesis and release of 5-HT and a reduced rate of firing of 5-HT neurons (17). A significant drop in rat core temperature was recorded as early as the first 15 min following microinjection of 5-HT into the DRN, the 5-HT_{1A} receptor subtype seeming to mediate this response (21). Recent experimental results obtained in our laboratory support the hypothesis of a major role played by the 5-HT_1 receptor, at the level of the DRN in the midbrain, in MSO-elicited hypothermia in the rat.

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