

Effects of Intrathecal Morphine, Clonidine, and Midazolam on the Somato-sympathoadrenal Reflex Response in Halothane-anesthetized Cats

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Modulatory effects of spinal opioid, α -adrenergic, and benzodiazepine receptors on the somato-sympathoadrenal reflex response, evoked by supramaximal bilateral sciatic nerve stimulation (50 times minimal motor threshold) were evaluated in halothane-anesthetized cats. Group 1 (n = 8) served as a control; group 2 (n = 7) received intrathecally (it) the opioid receptor agonist morphine (500 μ g); group 3 (n = 7), the α_2 -adrenergic agonist clonidine (200 μ g it); and group 4 (n = 7), the benzodiazepine receptor agonist midazolam (1 mg it). Plasma samples were collected from the adrenal vein at baseline, after it drug administration, and during sciatic nerve stimulation for the measurement of norepinephrine, epinephrine, and dopamine. In control cats (group 1), sciatic nerve stimulation evoked significant increases in adrenal vein catecholamine plasma levels, blood pressure, and heart rate. Morphine (group 2) did not have any effect on spontaneous hemodynamics and adrenal secretion. During stimulation (group 2), there were no significant increases in adrenal norepinephrine and epinephrine concentrations, whereas dopamine concentrations, blood pressure, and heart rate rose significantly. Clonidine (group 3) led to a decrease in heart rate and adrenal vein norepinephrine and epinephrine concentrations at baseline. During sciatic nerve stimulation in this group (3), significantly lower concentrations were observed in adrenal vein catecholamines compared with control, whereas the hemodynamic response was not suppressed. Midazolam suppressed baseline and stimulation-evoked adrenal vein catecholamine concentrations, but hemodynamics were not significantly affected. Although the study was conducted in a halothane-anesthetized cat model, employing single doses of agonists, the data clearly indicate a complex modulation of the somato-sympathoadrenal reflex response that is differentially inhibited by spinal opioid, benzodiazepine, and α -adrenergic receptor systems. (Key words: Anesthetics, hypnotics: midazolam. Anesthetics, opioids: morphine. Stimulation: nociceptive; sciatic nerve. Sympathetic nervous system, α_2 -adrenergic agonist: clonidine. Sympathetic nervous system, catecholamines: norepinephrine; epinephrine; dopamine.)

PAIN-EVOKED STIMULATION of the sympathoadrenal system is a major component of the endocrine response to surgery, which is characterized by a catecholamine (norepinephrine, epinephrine)-mediated hemodynamic

and metabolic response.¹ Although the physiologic importance of circulatory dopamine is not fully known, plasma dopamine presents a component of the normal stress response, as well as of stress-related diseases.² Modern anesthetic techniques aim to decrease the surgically evoked stress response based on the idea that intraoperative and postoperative mortality and morbidity may be reduced, and that the recovery period may be shortened and overall outcome improved.^{3,4} The optimal balance of suppression and stimulation of the different components of the neuroendocrine response to surgery has not been defined. Studies examining the pharmacologic modulation of the surgical stress response provide insight into the organization of pain-evoked reflex responses and are the basis for further clinical approaches to modulating individual components of this reflex response.

Several lines of evidence suggest that the pain-evoked activation of the sympathetic nervous system may be modulated at the level of the spinal cord by a variety of local receptor systems, including those for opioid, noradrenergic, and benzodiazepine agonists. Opioid receptors, present in high concentrations in the dorsal horn of the spinal cord, are associated presynaptically and postsynaptically with small primary afferents.⁵ Their occupation by opioid agonists selectively inhibits nociceptive afferent input onto dorsal horn neurons. Opioid receptors may potentially modulate preganglionic sympathetic outflow by action on sympathetic neurons in the intermediolateral cell column.⁶ Certain bulbospinal pathways contain and release norepinephrine in response to peripheral and central stimulation.⁷ Norepinephrine, by a local spinal action, exerts inhibitory effects on nociceptive processing, mediated by α -adrenoreceptors located presynaptically and postsynaptically to primary afferents.⁸ Sympathetic preganglionic outflow may be directly inhibited by an α_2 -adrenoreceptor-mediated action onto cells in the intermediolateral cell column.⁹ Benzodiazepines, acting on the GABA (gamma-aminobutyrate) receptor-ionophore complex on primary afferents in the dorsal horn of the spinal cord,¹⁰ depress primary afferent input by presynaptic inhibition.¹¹ Further, benzodiazepines might act on or near sympathetic preganglionic neurons, as specific benzodiazepine-binding sites have been identified in the intermediolateral cell column.¹⁰ The present experiments examined potential modulatory effects of intrathecally (it)

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administered morphine (opioid receptor agonist), clonidine (α_2 -adrenergic receptor agonist), and midazolam (central benzodiazepine receptor agonist) on the hemodynamic and adrenal catecholamine response evoked by high-intensity sciatic nerve stimulation in halothane-anesthetized cats.

Materials and Methods

Experiments were performed after approval of the Mayo Foundation Animal Care Committee. Twenty-nine mongrel cats (mean body weight \pm SE: 3.5 ± 0.2 kg) of either sex (18 males, 11 females) were included in the study. In the morning after a 12-h fasting period with *ad libitum* access to water, anesthesia was induced in an airtight plexiglass box with 4 vol% halothane in an air/oxygen mixture. After tracheal intubation at a deep level of anesthesia, animals were artificially ventilated with a halothane/oxygen mixture (Harvard animal ventilator) while the end-tidal halothane concentration was continuously measured and kept at 1.6 vol% during the preparation and at 0.8 vol% during the course of the experiment. Arterial blood gases were determined after termination of the surgical procedure, and ventilator settings were adjusted to keep values within normal limits (pH: 7.35 ± 0.05 ; P_{CO_2} : 36 ± 5 mmHg; P_{O_2} : 90–150 mmHg).¹² Rectal temperature was continuously measured and kept between 37° and 38° C by means of a heating pad. A femoral artery and vein were cannulated with PE-90 catheters to allow continuous measurement of blood pressure and heart rate, and administration of drugs. Cannulation of the left adrenal vein was performed with a 22-G Teflon cannula, according to a technique described by Hume and Nelson,¹³ which allows selective collection of blood from the adrenal while preserving the splanchnic innervation.

To avoid clotting of blood in catheters, heparin was administered as an initial bolus of 200 U/kg intravenously (iv), followed by 100 U/kg iv every 2 h. The sciatic nerves were isolated bilaterally at the level of the sciatic notch, bathed in oil, and placed on bipolar hook electrodes that were implanted in dental cement to avoid stimulation of surrounding tissue. The minimum motor threshold, corresponding to the threshold current that evoked a clear motor twitch of the hind paw, was defined at either nerve by increasing the voltage with otherwise constant stimulation variables: 50 Hz, 0.5 ms, train duration 1 sec, train rate 0.5/s (Grass S-88 stimulator, PSIU-6 constant-current isolation units). Pancuronium bromide ($0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ iv) was administered after determination of the minimum motor threshold to achieve muscle relaxation throughout the experiment. Placement of it lumbar catheters was performed with the head placed in a stereotactic frame. A PE10 catheter was inserted

through the atlanto-occipital membrane and advanced intrathecally 30 cm caudally to position its tip at the lumbar enlargement of the spinal cord.¹⁴ The correct catheter placement was verified at the end of the experiment by dye injection.

DRUG ADMINISTRATION

Cats were divided in random order into four groups according to the drugs administered. Group 1 ($n = 8$) served as a control and received no drug. Group 2 ($n = 7$) received the opioid agonist morphine (500 μg it), group 3 ($n = 7$) the α_2 receptor agonist clonidine (200 μg it), and group 4 ($n = 7$) received the benzodiazepine agonist midazolam (1 mg it). Morphine sulfate and clonidine hydrochloride (Boehringer Ingelheim) were freshly prepared in 0.9% NaCl, where they were freely soluble. Midazolam (Roche), purchased in liquid form, was lyophilized and then diluted in 0.9% NaCl. The preservatives of injectable midazolam are benzyl alcohol and EDTA. Benzyl alcohol is lost during the lyophilization procedure. Injected it, it has no effect on the somato-sympathetic reflex response.¹⁵ Only small amounts of EDTA were injected compared with midazolam (20 μg EDTA/1 mg midazolam). The it injection of EDTA (Sigma; 300 μl) in rabbits ($n = 3$) led to a transient increase in blood pressure from 108 ± 11 mmHg to 121 ± 13 mmHg (mean \pm SE) in the first 5 min to return shortly thereafter to preinjection levels. § Thus, it is reasonably certain that the observed drug effects were not related to the vehicle. Intrathecal drugs were always administered in a volume of 300 μl followed by a saline flush of 150 μl . Doses were selected on the basis of significant suppression of nociceptive reflexes and relative potencies in other animal models, as such data do not exist in the feline model. Thus, administration of morphine it at a dose of 500 μg lies in the range of dosages that provide powerful spinal analgesia in cats.¹⁶ Intrathecal clonidine (200 μg), on a dose per kilogram body weight basis, was above doses reported to inhibit the thermally evoked nociceptive response in rats.¹⁷ Midazolam was administered at a dose (1 mg it) previously reported to suppress the nociceptive sympathetic reflex in dogs.¹⁵ The practical justification of the doses selected was given by the results, in that all agonists evoked inhibitory effects.

EXPERIMENTAL PROTOCOL

During the course of the experiment, bilateral sciatic nerve stimulation was employed over a 10-min period in all cats, at high intensities, with currents of 50 times minimal motor threshold, previously shown to consistently

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evoke stimulation of slowly conducting A δ - and C-fibers,⁷ mediating nociceptive information. On an average, the voltage employed to either nerve was 30 ± 4 V (mean \pm SE). At specific time points of the experiments, blood samples were taken from the adrenal vein by syringe pump withdrawal ($500 \mu\text{l}/\text{min}$) during 10-min periods. In all animals, the baseline sample S1 was taken 1 h after termination of the surgical procedure. In group 1, this was followed by a 10-min stimulation period of both sciatic nerves. In groups 2, 3, and 4, baseline sample S1 was followed by it administration of either morphine, clonidine, or midazolam, respectively. Sample S2 was collected in these three groups 10 min after it drug administration, and the sample was immediately followed by a 10-min period of sciatic nerve stimulation during which interval sample S3 was collected. At the end of the experiments, animals were killed by an overdose of pentobarbital.

SAMPLE COLLECTION AND ASSAYS

Blood samples from the adrenal vein were collected in syringes that were flushed with EDTA and that were covered by ice. The collected blood was continuously replaced by an infusion of the same volume of hetastarch into the femoral vein. Samples were immediately centrifuged in precooled tubes at 4°C . Plasma was pipetted in tubes containing freshly prepared sodium metabisulfite ($10 \mu\text{l}$ of a 5% solution per milliliter plasma). Catecholamine concentrations in plasma were measured by high-performance liquid chromatography with electrochemical detection.¹⁸ The assay sensitivity (defined as signal-to-noise ratio > 2) was 4 pg for norepinephrine, 5 pg for epinephrine, and 6 pg for dopamine as injected on the column. The practical limit of sensitivity, assuming 1 ml of plasma was assayed, was 0.07 ng/ml for norepinephrine, 0.09 ng/ml for epinephrine, 0.11 ng/ml for dopamine, and 0.07 ng/ml for dihydroxybenzylamine, which was run as an internal standard with each sample. Mean recoveries were 85% for norepinephrine and dopamine and 82% for epinephrine. Data were corrected for these recoveries.

STATISTICAL ANALYSIS

Group differences in body weight were analyzed by one-way analysis of variance (ANOVA), differences in sex distribution by the chi-square test. Hemodynamic and hormonal variables were analyzed in each treatment group. Analysis of adrenal vein catecholamine levels was performed after transformation to logarithms to establish homogeneity of variance. In those cases, in which baseline values were below the assay sensitivity, the midpoint between zero and assay sensitivity was taken as estimated baseline value. Within-group differences were detected by *a priori* paired Student's *t* test, carried out in control animals between baseline values and subsequent sciatic

stimulation, and in groups 2, 3, and 4 between S1 and S2, and S2 and S3. A Bonferroni adjustment for multiple comparisons was applied in groups 2, 3, and 4. Between-group differences were detected by one-way ANOVA in comparing the following time points: S1 in all groups, S1 (group 1) and S2 (groups 2, 3, and 4), and S3 in all groups. If the ANOVA main effects were significant, between-group differences were detected by *post hoc* comparisons using the Duncan multiple range test. All tests were two-tailed and evaluated at the 5% level of significance. In view of the variance of data distribution and the number of animals examined, the sensitivity of the statistical tests employed was limited.

Results

The different experimental groups did not differ with regard to body weight and sex distribution. Because of the fact that mongrel cats were used, no exact data on the age of the animals was available. However, based on data on cross-bred European domestic and Abyssinian cats, cats were generally older than 7 months.[†]

At baseline, cats in the different treatment groups (2, 3, and 4) did not differ from control (group 1) with regard to body weight, hemodynamics, and adrenal vein catecholamine plasma concentrations (table 1). Highest levels were observed for epinephrine, followed by norepinephrine and dopamine, in decreasing order.

The effects of it administration of morphine ($500 \mu\text{g}$), clonidine ($200 \mu\text{g}$), and midazolam (1 mg) on adrenal vein catecholamine levels as well as on hemodynamic variables are shown in figure 1. Preliminary experiments in an identical cat model showed no effect of it saline injection on adrenal vein catecholamine levels and hemodynamics.^{**} The it administration of morphine or midazolam did not cause any changes in blood pressure, heart rate, and catecholamine levels. In contrast, it administration of clonidine evoked a significant reduction in heart rate compared with baseline (134 ± 5 beats/min *versus* 223 ± 7 beats/min, mean \pm SE) as well as a decrease in adrenal vein norepinephrine and epinephrine levels, representing $1/5$ and $1/8$, respectively, of baseline values.

SCIATIC NERVE STIMULATION

The effects of sciatic nerve stimulation on hemodynamic variables are shown in figure 2, and on adrenal vein catecholamine levels in figure 3. In control animals (group 1), sciatic nerve stimulation evoked a significant increase in mean arterial blood pressure (173 ± 8 mmHg, mean \pm SE) and heart rate (274 ± 17 beats/min) as com-

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TABLE 1. Hemodynamics and Plasma Levels at Baseline During Halothane Anesthesia in the Four Experimental Groups of Cats

Group	Hemodynamics	MABP (mm Hg)	HR (bpm)	NE (ng/ml)	EPI (ng/ml)	DA (ng/ml)
1	Control (n = 8)	110 ± 7	215 ± 12	3.24 (2.05, 5.12)	11.5 (8.32, 15.8)	0.18 (0.12, 0.27)
2	Morphine (n = 7)	129 ± 4	204 ± 11	4.47 (1.82, 10.95)	26.3 (11.7, 58.9)	0.31 (0.18, 0.53)
3	Clonidine (n = 7)	134 ± 6	223 ± 7	1.95 (1.05, 3.63)	6.17 (2.88, 13.2)	0.09 (0.07, 0.11)
4	Midazolam (n = 7)	113 ± 10	194 ± 10	0.81 (0.46, 1.44)	5.89 (2.45, 14.1)	0.09 (0.08, 0.10)

Mean arterial blood pressure (MABP), heart rate (HR) (mean ± SE), and adrenal vein plasma levels (geometric means with limits of interval

= geometric mean × ÷ geometric SE) for norepinephrine (NE), epinephrine (EPI), and dopamine (DA) before drug treatment.

pared with baseline values (110 ± 7 mmHg; 215 ± 12 beats/min). Adrenal vein catecholamine levels rose significantly: fivefold for epinephrine, fourfold for norepinephrine, and twofold for dopamine.

Following it morphine (group 2), the absolute hemodynamic (fig. 2) and adrenal secretory response (fig. 3)

during sciatic nerve stimulation were not suppressed as compared with control (group 1). However, the adrenal vein norepinephrine and epinephrine plasma levels did not increase significantly above prestimulation values.

After it clonidine, despite an initial decrease in heart rate, the hemodynamic response during sciatic nerve stimulation in this group (group 3; fig. 2) was not different from control (group 1). The inhibitory effect of it clonidine on resting adrenal secretion was manifested in unstimulated animals by a significant reduction in adrenal vein norepinephrine and epinephrine levels (fig. 1). Thus, during sciatic stimulation, adrenal vein plasma levels of norepinephrine, epinephrine, and dopamine (fig. 3) were significantly lower than in control animals.

Clonidine, however, did not suppress the dynamic response of adrenal norepinephrine and epinephrine secretion, as stimulation continued to evoke significant increases of these hormones as compared with prestimulation values. During stimulation, adrenal vein norepinephrine and epinephrine values rose 5.6- and 13-fold, respectively, in comparison with fourfold and fivefold increases in non-drug-treated controls.

Intrathecal midazolam (group 4), similar to morphine and clonidine, did not suppress the increase in mean arterial blood pressure and heart rate, evoked by sciatic nerve stimulation (fig. 2). However, a significant suppression in the sympathoadrenal secretory response was observed in midazolam-treated cats during sciatic nerve stimulation, with adrenal catecholamine levels being significantly lower than in control cats (group 1, fig. 3). Prestimulation values of adrenal vein norepinephrine and epinephrine levels were significantly lower in midazolam-treated cats (group 4) than in control cats (group 1), although it midazolam administration did not cause significant sympatho-inhibitory effects in unstimulated cats (fig. 1). This reflects numerically lower values of norepinephrine and epinephrine levels in the adrenal vein at baseline, before drug administration, in group 4 versus baseline values in group 1. Independent of lower prestimulation values, no significant increases in adrenal vein catechol-

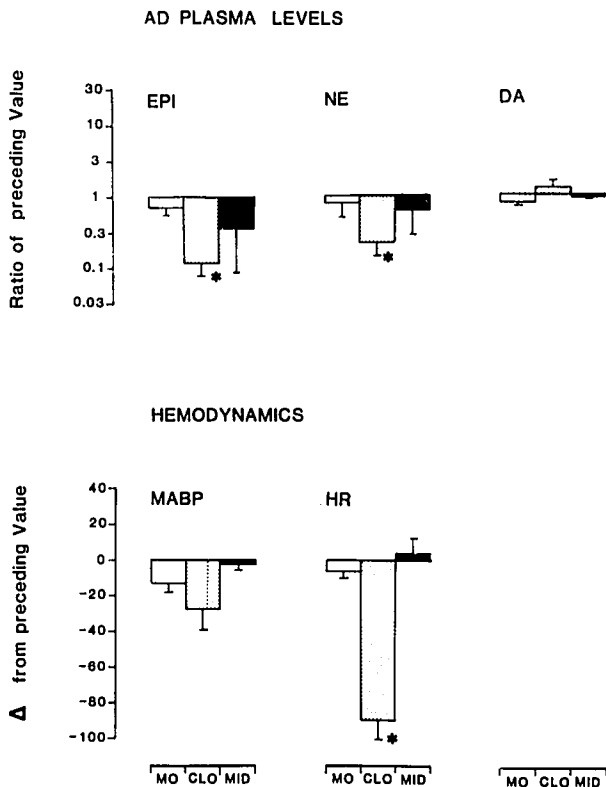


FIG. 1. (Top) Plasma levels of norepinephrine (NE), epinephrine (EPI), and dopamine (DA) expressed as ratios of baseline values (geometric mean ± geometric SE) in adrenal vein (AD) in cats that had received either morphine (MO; 500 µg it; n = 7), clonidine (CLO; 200 µg it; n = 7), or midazolam (MID; 1 mg it; n = 7). (Bottom) Mean arterial blood pressure (MABP) and heart rate (HR) expressed as change (Δ) from baseline values in same groups of cats as described. *P < 0.05 compared to preceding value.

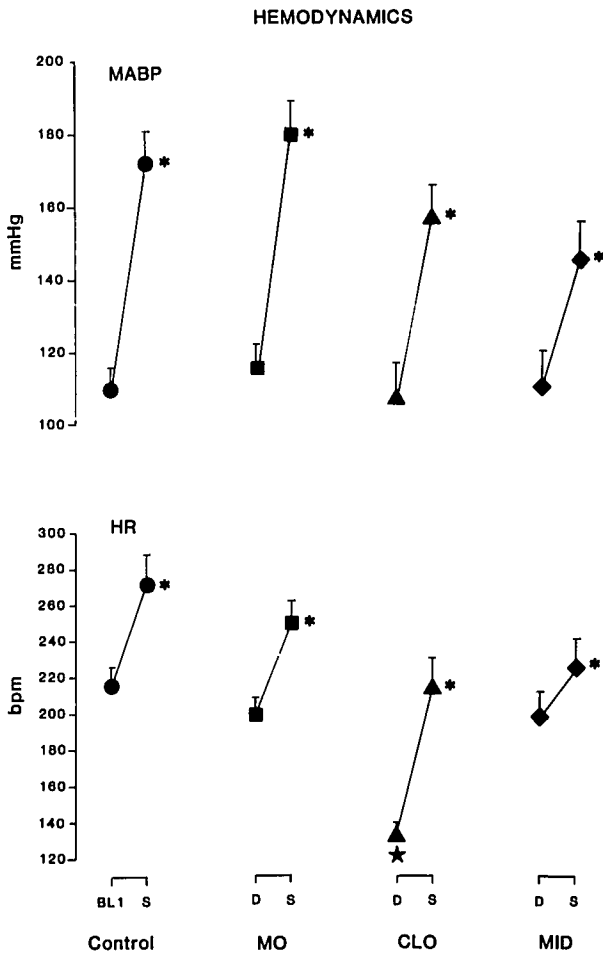


FIG. 2. Mean arterial blood pressure (MABP) and heart rate (HR) (mean \pm SE) in four groups of cats, one control, and three treatment groups. In the control group (n = 8), data are presented at baseline (BL1) and during subsequent sciatic nerve stimulation (S). In the treatment groups, data are presented for values obtained 10 min after drug administration (D) and during sciatic nerve stimulation (S). Drugs administered in the MO group (n = 7) were morphine (500 μ g it); in the CLO group (n = 7), clonidine (200 μ g it) and in the MID group (n = 7), midazolam (1 mg it). **P* < 0.05 compared to preceding value. **P* < 0.05 compared to control group.

amine levels were observed during stimulation in midazolam-treated cats.

Discussion

The present study examined the pharmacologic modulation of the pain-evoked sympathoadrenal response by the spinal administration of selective opioid-, α -adrenergic-, and benzodiazepine receptor agonists. These different groups of drugs are of great clinical importance, as they are widely employed by systemic and/or spinal/epidural routes in anesthesiologic practice. Because the degree of pain intensity is not necessarily closely related to the neuroendocrine stress response, and because preop-

erative and intraoperative stress may have long-lasting sequelae,^{3,4} the selective comparison of the pharmacologic modulation of different components of the surgical stress response is necessary. Animal studies provide the basis for the understanding of the complex organization of the stress response to surgery.

ANIMAL MODEL

For ethical reasons, experiments were conducted under general anesthesia. A light level of halothane anesthesia (0.8 vol%) was employed, not exceeding the MAC (minimum alveolar concentration) value.^{19,20} This level of anesthesia is below the level that blocks pain-evoked, sympathetic stimulation,²¹ but probably inhibits the sympathetic response to a certain degree, because of various mechanisms. Thus, halothane has been shown to inhibit adrenal catecholamine storage mechanisms²² and nicotinic receptor-mediated secretion,²³ and to decrease sympathetic ganglionic transmission.²⁴ Halothane suppresses the

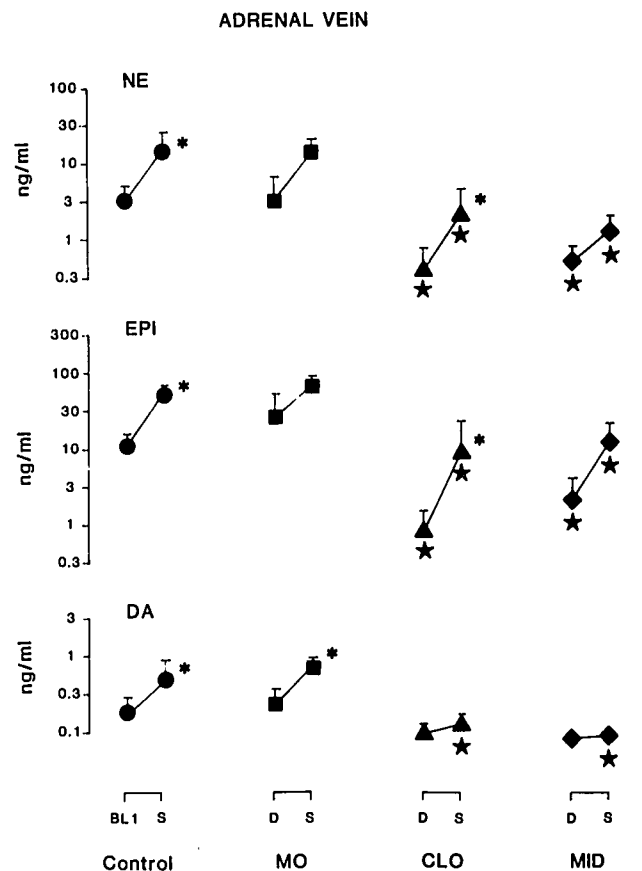


FIG. 3. Adrenal vein plasma levels of norepinephrine (NE), epinephrine (EPI), and dopamine (DA) (geometric mean \pm geometric SE), in four groups of cats during the experiment, corresponding to data presented in Fig. 2. Data points and abbreviations are the same as described in Fig. 2. **P* < 0.05 compared to preceding value. **P* < 0.05 compared to control group.

baroreceptor reflex²⁵ and the spinal transmission of noxious afferent input.²⁶ The individual components of the various reflex responses show different degrees of susceptibility to the suppressant effects of halothane.²⁵

The muscle relaxant pancuronium bromide ($1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ iv) was employed, to achieve control over ventilation and to avoid spontaneous and stimulation-evoked movements. A recent clinical study showed no effects of pancuronium on the MAC value of halothane.²⁷ Although it morphine, clonidine, and benzodiazepines may reduce anesthetic requirements (MAC) to different degrees, no efforts were made to address this specific question, as MAC-reducing effects of these drugs will be reflected in the overall somatosympathetic reflex response. High-intensity sciatic nerve stimulation (50 times minimal motor threshold) was employed to activate slowly conducting A δ - and C-fibers, mediating nociceptive information. At these intensities, faster conducting, low-threshold A α - and A β -primary afferents, mediating non-painful sensations are also activated.²⁸ However, stimulation of A α - and A β primary afferents alone does not evoke sympathetic stimulation.²⁹

Sciatic nerve stimulation in control animals evoked significant increases in mean arterial blood pressure, heart rate, and adrenal vein catecholamine plasma levels. However, increases in adrenal vein catecholamine levels were moderate (approximately tenfold less) as compared with those observed during severe hemorrhage or splanchnic artery occlusion shock in a comparable cat model.^{30,31} This may indicate that nociceptive afferent input in the halothane-anesthetized cat is less effectively coupled to preganglionic sympathetic neurones in the intermediolateral cell column than is baroreceptor stimulation.

EFFECTS OF INTRATHECAL MORPHINE, CLONIDINE, AND MIDAZOLAM

During sciatic nerve stimulation, cats treated with it morphine at a dose sufficient to cause spinal analgesia¹⁶ showed no suppression in adrenal norepinephrine and epinephrine levels as compared with control, although no significant increase in these levels from prestimulation values were observed. The relatively small effect of it morphine on afferent-evoked adrenal secretion and the failure to block the stimulus-evoked hemodynamic response is surprising in view of the selective inhibitory effects of morphine on spinal pain-transmitting processes.⁵ The small effect of it morphine on the somato-sympathoadrenal reflex response might be explained by the differential actions of opioids on elements of spinal nociceptive transmission. Thus, morphine suppresses dorsal horn neurons, excited by C-fiber afferents, to a greater degree than those excited by A δ -afferents,³² and fentanyl suppresses dorsal horn wide dynamic range neurons to a greater degree than high-threshold neurons.³³ In rats,

even high doses of iv morphine (10 mg/kg) did not suppress the somatosympathetic reflex evoked by hind paw pinching, whereas a lower dose of morphine (1 mg/kg) completely blocked this reflex when it was evoked by lower chest pinching.³⁴ This indicates a differential connectivity of the body dermatomes to afferent drive of sympathetic neurons in the intermediolateral cell column. Alternatively, in this model, there may be already a high level of existing central opioid drive, which is not much further intensified by it morphine, as high-intensity sciatic nerve stimulation evokes the spinal and supraspinal release of Met-enkephalin.³⁵

Administration of it clonidine significantly suppressed spontaneous sympathetic activity, as evidenced by a reduction in adrenal vein norepinephrine and epinephrine levels. Based on its pharmacology, this is most likely due to α_2 -receptor-mediated inhibitory effects on preganglionic sympathetic neurons,⁹ leading to a reduction in spontaneous splanchnic nerve discharge, as has been observed following it clonidine administration in rats.³⁶ Mean arterial blood pressure was not significantly reduced, corresponding to observations in sheep in which epidurally administered clonidine did not cause hypotension.³⁷ A highly significant reduction in heart rate, however, was observed after it clonidine. This suggests a cephalad spread of the spinally administered drug indicating a higher susceptibility of sympathetic preganglionic cardiac neurons *versus* vasomotor neurons to the effects of it clonidine, and/or activation of cardio-inhibitory brain stem sites.³⁸ Intrathecal clonidine during sciatic stimulation suppressed neither the hemodynamic response nor the dynamics of adrenal medullary secretion. This is consistent with observations that spontaneous sympathetic discharge is more readily suppressed than stimulation-induced discharge.³⁹

Intrathecal administration of midazolam (1 mg) led to a significant reduction in absolute levels of norepinephrine and epinephrine in adrenal vein as compared with control cats, but had no hemodynamic suppressant effects. In dog experiments, the same dose of it midazolam (1 mg) did not evoke any changes in hemodynamics or renal sympathetic nerve discharge.¹⁵ The difference in efferent sympathetic activity to the adrenal (in cats) and renal nerve (in dogs) might be explained on the basis of higher doses per body weight of it midazolam in the present experiments. During sciatic nerve stimulation, it midazolam-treated cats showed a significant reduction in adrenal vein catecholamine levels as compared with control cats. In contrast to clonidine-treated cats, it midazolam reduced the dynamics of the sympathoadrenal reflex response, as evidenced by insignificant changes from prestimulation to stimulation values in adrenal vein norepinephrine and epinephrine levels. This observation indicates an association of spinal benzodiazepine receptors with pathways

mediating the adrenal secretory response to nociceptive stimulation^{10,11} and corresponds to dog experiments that showed a selective midazolam-mediated spinal suppression of the nociceptive sympathetic reflex.¹⁵ It further corresponds to clinical observations in which systemically applied benzodiazepines reduced intraoperative or postoperative catecholamine levels.⁴⁰ However, also in midazolam-treated cats, blood pressure and heart rate response evoked by sciatic nerve stimulation were not significantly suppressed.

To summarize, the present study indicates that it midazolam, a benzodiazepine agonist, suppresses adrenal catecholamine secretion during resting conditions and nociceptive afferent stimulation. Intrathecal clonidine, an α -adrenergic agonist, preferentially inhibits spontaneous sympathetic activity, whereas morphine, an opioid receptor agonist, mildly suppresses stimulation-evoked adrenal catecholamine secretion. None of the spinally administered agonists significantly suppressed the spontaneous and the stimulation-evoked blood pressure response. This indicates the lack of a close correlation between hemodynamic response and adrenal catecholamine secretion. It further indicates, by exclusion, the importance of supraspinal sites in mediating hemodynamic suppressant effects of these different receptor agonists.

Conclusions from this study are limited by the species, general anesthetic, and single doses of agonists employed. Halothane may have blunted the pain-evoked sympathoadrenal reflex response. High doses of agonists employed assure pharmacologic effects, but bear the risk of increased nonspecific effects. Despite these limitations, the data clearly show the complex pharmacologic organization of the somatosympathetic reflex response to a well-defined painful stimulus.

The human sympathoadrenal response to surgery, in contrast, may be markedly influenced by the nature of the intervention and the dermatomes affected, and not just by the stimulus intensity.

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References

1. Halter JB, Pflug AE, Porte D: Mechanism of plasma catecholamine increases during surgical stress in man. *J Clin Endocrinol Metab* 45:936-939, 1977
2. Snider ST, Kuchel O: Dopamine: An important neurohormone of the sympathoadrenal system. Significance of increased peripheral dopamine release for the human stress response and hypertension. *Endocr Rev* 4:291-309, 1983
3. Kehlet H: Modification of responses to surgery by neural blockade: Clinical implications, *Neural Blockade in Clinical Anesthesia and Management of Pain*. Edited by Cousins MJ, Bridenbraugh PO. Philadelphia, JB Lippincott, 1988, pp 145-188
4. Wall PD: The prevention of postoperative pain. *Pain* 33:289-290, 1988
5. Fields HL, Emson PC, Leigh BK, Gilbert RFT, Iversen LL: Multiple opiate receptor sites on primary afferent fibres. *Nature* 284:351-353, 1980
6. Romagnano MA, Hamill RW: Spinal sympathetic pathway: An enkephalin ladder. *Science* 255:737-739, 1984
7. Tyce GM, Yaksh TL: Monoamine release from cat spinal cord by somatic stimuli: An intrinsic modulatory system. *J Physiol (Lond)* 314:513-529, 1981
8. Yaksh TL: Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. *Pharmacol Biochem Behav* 22:845-858, 1985
9. Guyenet PG, Cabot JB: Inhibition of sympathetic preganglionic neurons by catecholamines and clonidine: Mediation by an alpha-adrenergic receptor. *J Neurosci* 1:908-917, 1981
10. Faull RLM, Villiger JW: Benzodiazepine receptors in the human spinal cord: A detailed anatomical and pharmacological study. *Neuroscience* 17:791-802, 1986
11. Levy RA: The role of GABA in primary afferent depolarization. *Prog Neurobiol* 9:211-267, 1977
12. Ringler DH, Peter GK: Dogs and cats as laboratory animals, *Laboratory Animal Medicine*. Edited by Fox JG, Cohen BJ, Loew FM. Orlando, Academic Press, 1984, pp 241-271
13. Hume DM, Nelson DH: Adrenal cortical function in shock. *Surg Forum* 5:568-575, 1955
14. Yaksh TL: Analgesic actions of intrathecal opiates in cat and primate. *Brain Res* 153:205-210, 1978
15. Niv D, Whitwam JG, Loh L: Depression of nociceptive sympathetic reflexes by the intrathecal administration of midazolam. *Br J Anaesth* 55:541-547, 1983
16. Yaksh TL, Noueihed RY, Durant PAC: Studies of the pharmacology and pathology of intrathecally administered 4-anilino-piperidine analogues and morphine in the rat and cat. *ANESTHESIOLOGY* 64:54-66, 1986
17. Yasuoka S, Yaksh TL: Effects on nociceptive threshold and blood pressure of intrathecally administered morphine and α -adrenergic agonists. *Neuropharmacology* 22:309-315, 1983
18. Bove AA, Dewey DJ, Tyce M: Increased conjugated dopamine in plasma after exercise training. *J Lab Clin Med* 104:77-85, 1984
19. Brown BR, Crout JR: A comparative study of the effects of five general anesthetics on myocardial contractility. *ANESTHESIOLOGY* 34:236-245, 1971
20. Steffey EP, Gillespie JR, Berry JD, Eger II ET, Munson ES: Anesthetic potency (MAC) of nitrous oxide in the dog, cat, and stump-tail monkey. *J Appl Physiol* 36:530-533, 1974
21. Roizen MF, Horrigan RW, Frazer BM: Anesthetic doses blocking adrenergic (stress) and cardiovascular responses to incision—MAC BAR. *ANESTHESIOLOGY* 54:390-398, 1981
22. Sumikawa K, Amakata Y, Yoshikawa K, Kashimoto T, Izumi F: Catecholamine uptake and release in isolated chromaffin granules exposed to halothane. *ANESTHESIOLOGY* 53:385-389, 1980
23. Sumikawa K, Matsumoto T, Ishizaka N, Nagai H, Amenomori Y, Amakata Y: Mechanism of the differential effects of halothane on nicotinic and muscarinic-receptor-mediated responses of the dog adrenal medulla. *ANESTHESIOLOGY* 57:444-450, 1982
24. Bosnjak J, Seagard JL, Wu A, Kampine JP: The effects of halothane on sympathetic ganglionic transmission. *ANESTHESIOLOGY* 57:473-479, 1982
25. Seagard JL, Hopp FA, Donegan JH, Kalbfleisch JH, Kampine JP: Halothane and the carotid sinus reflex. *ANESTHESIOLOGY* 57:191-202, 1982

26. Namiki A, Collins JG, Kitahata LM, Kikuchi H, Homma E, Thahammer JG: Effects of halothane on spinal neuronal responses to graded noxious heat stimulation in the cat. *ANESTHESIOLOGY* 53:475-480, 1980
27. Fahey MR, Sessler DI, Cannon JE, Brady K, Stoen R, Miller RD: Atracurium, vecuronium and pancuronium do not alter the minimum alveolar concentration of halothane in humans. *ANESTHESIOLOGY* 71:53-56, 1989
28. Sato A, Sato Y, Schmidt RF: Heart rate changes reflecting modifications of efferent cardiac sympathetic outflow by cutaneous and muscle afferent volleys. *J Auton Nerv Syst* 4:231-247, 1981
29. Sato A, Sato Y, Schmidt RF: Catecholamine secretion and adrenal nerve activity in response to movements of normal and inflamed knee joints in cats. *J Physiol (Lond)* 375:611-624, 1986
30. Gaumann DM, Yaksh TL: Adrenal and intestinal secretion of catecholamines and neuropeptides during splanchnic artery occlusion shock. *Circ Shock* 26:391-407, 1988
31. Gaumann DM, Yaksh TL, Tyce GM, Lucas DL: Opioids preserve the adrenal medullary response evoked by severe hemorrhage: Studies on adrenal catecholamine and Met-enkephalin secretion in halothane anesthetized cats. *ANESTHESIOLOGY* 68:743-753, 1988
32. Duggan AW, North RA: Electrophysiology of opioids. *Pharmacol Rev* 35:219-281, 1984
33. Kumeta Y, Murata K, Kitahata LM, Aoki M, Nishio Y, Collins JG: Fentanyl suppression of nociceptive neurons in the superficial dorsal horn of the cat. *ANESTHESIOLOGY* 69:371-376, 1988
34. Togashi H, Kurosawa M, Minami M, Saito H: Effect of morphine on the adrenal sympathetic reflex elicited by mechanical stimulation of the skin in rats. *Neurosci Lett* 62:81-87, 1985
35. Jhamandas K, Yaksh TL, Go VLW: Acute and chronic morphine modifies the in vivo release of methionine enkephalin-like immunoreactivity from the cat spinal cord and brain. *Brain Res* 297:91-103, 1984
36. LoPachin RM, Rudy TA: The effects of intrathecal sympathomimetic agents on neural activity in the lumbar sympathetic chain of rats. *Brain Res* 224:195-198, 1981
37. Eisenach JC, Dewan DM, Rose JC, Angelo JM: Epidural clonidine produces antinociception, but not hypotension, in sheep. *ANESTHESIOLOGY* 66:496-501, 1987
38. Unnerstall JR, Kopajtic TA, Kuhar MJ: Distribution of α_2 -agonist binding sites in the rat and human central nervous system: Analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. *Brain Res Rev* 7:69-101, 1984
39. Kobinger W: Central α -adrenergic systems as targets for hypotensive drugs. *Rev Physiol Biochem Pharmacol* 81:40-100, 1978
40. Crozier TA, Beck D, Schlaeger M, Wuttke W, Kettler D: Endocrinological changes following etomidate, midazolam, or methohexital for minor surgery. *ANESTHESIOLOGY* 66:628-635, 1987