

Opioids Preserve the Adrenal Medullary Response Evoked by Severe Hemorrhage: Studies on Adrenal Catecholamine and Met-Enkephalin Secretion in Halothane Anesthetized Cats

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Possible modulatory effects of μ -, δ -, and κ -receptor agonists on the concurrent adrenal secretion of catecholamines and met-enkephalin evoked by staged hemorrhage were examined in four groups of cats ($n = 5$ in each group) anesthetized with halothane (1 MAC). Group I received saline, group II received the μ -agonist sufentanil (25 $\mu\text{g}/\text{kg}$ i.v., followed by a maintenance infusion), group III received the δ/μ agonist metkephamid (3 mg/kg i.v.), and group IV the κ agonist U50488H (3.5 mg/kg i.v.). Samples for norepinephrine, epinephrine, dopamine, and met-enkephalin were taken simultaneously from the adrenal vein, femoral vein, and femoral artery at baseline, after drug administration, and after induction of 25% and 50% hemorrhage. In cats receiving saline, 25% hemorrhage resulted in a significant decline in mean arterial blood pressure (MABP) and no change in adrenal secretion. Fifty percent hemorrhage evoked no significant further fall in MABP, but led to prominent increases in adrenal vein hormone levels (norepinephrine, 30-fold; dopamine, 14-fold; and epinephrine, ten-fold) as compared to post-saline values. During the pre-hemorrhage baseline state, administration of sufentanil evoked a significant six- to 20-fold rise in adrenal vein catecholamine and met-enkephalin levels, whereas the administration of metkephamid and U50488H produced no change in adrenal secretion and a decrease in MABP. After 25% and 50% hemorrhage, there was no difference in adrenal vein hormone levels in cats receiving the μ -, δ -, or κ -agonists compared to those receiving saline. No differences were observed in the different treatment groups with regard to the proportional levels of catecholamines and met-enkephalin in the adrenal vein during the course of the experiment. The authors conclude that opioids are not involved in the regulation of the secretory adrenal medullary response evoked by hemorrhage, and that the systems involved in mediating these cardiovascular reflexes differ pharmacologically from those systems mediating the autonomic response evoked by pain. (Key words: Opioids, agonists: metkephamid; Sufentanil; U50488H. Receptors, opioid: delta; kappa; mu. Reflex: baroreceptor. Shock, hemorrhagic: adrenal response.)

OPIOIDS REDUCE the autonomic response to painful stimulation as shown by a reduction in heart rate and blood pressure, as well as in catecholamine secretion during surgical procedures.¹ Apart from actions on spe-

cific receptor sites in brain and spinal cord,^{2,3} opioids may exhibit their inhibitory effects on the sympathetic response by direct actions on adrenal secretory mechanisms. In the adrenal medulla, opioids are present in a variety of forms, one of which is met-enkephalin, and are located in splanchnic nerve endings and in chromaffin cells.⁴ Stereospecific opioid receptor binding sites (μ , δ , and κ) have been localized on adrenal chromaffin cell membranes.^{5,6} By occupation of these receptors, opioids may lead to a decrease in nicotinic receptor sites on chromaffin cells and, thus, decrease the nicotinic evoked release of catecholamines.⁷ Opioids released with acetylcholine from splanchnic nerve terminals, and with catecholamines from chromaffin cells, may exert neuromodulatory functions on chromaffin cells, apart from their possible systemic hormonal effects. If opioids acted directly on adrenal secretory mechanisms, one would anticipate that their effects on the adrenal secretory response could be seen following adrenal activation by a variety of different physiological stimuli. The present study sought to examine the possible modulatory effects of selective opioid receptor agonists μ , δ , and κ , on the adrenal secretion of catecholamines and met-enkephalin evoked by staged hemorrhage.⁸ The following drugs were selected: sufentanil, metkephamid, and U50488H. Sufentanil is a highly selective μ -agonist, 600 to 700 times more potent than morphine, and possibly more potent in suppressing the surgically evoked autonomic response compared to other μ -agonists.⁹ Metkephamid (Tyr-D-Ala-Gly-Phe-N(Me)Met-NH₂) is a systemically active analog of met-enkephalin that has a high affinity for and efficacy of the δ -receptor. Though it may also exert an action on the μ -receptors, its affinity for δ -receptors is 12-fold higher than that of morphine,¹⁰ and its analgesic potency is comparable to meperidine.¹¹ U50488H (trans-3,4-dichloro-N-methylN-[2(1-pyrrolidiny)-cyclohexyl]benzeneacetamide, methanesulfonate) is a highly selective κ receptor agonist with a 150-fold greater affinity for κ than μ -receptors.¹² As an analgesic, it has been shown to be one-half as potent as morphine in a variety of analgesic tests in mice.¹³

Materials and Methods

Experiments were performed in 20 mongrel cats (mean body weight \pm SE: 3.2 \pm 0.2 kg) in the morning,

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after a 12-h fasting period with *ad lib* access to water. Experiments were conducted during halothane anesthesia with artificial ventilation and muscle relaxation (pancuronium bromide 0.1 mg/kg iv q 1 h). The end-tidal halothane concentration was continuously measured and anesthesia was maintained at 2 MAC during the preparation, and at 1 MAC (0.8 vol%) during the course of the experiment. The rectal temperature was continuously measured and kept between 37 and 38° C with a heating pad. Femoral arteries, the femoral vein, and the jugular vein were cannulated with PE-90 catheters to allow continuous measurement of blood pressure and heart rate, administration of drugs, collection of blood samples, and induction of hemorrhage. Arterial blood gases were intermittently examined to assure normoventilation. Cannulation (22-gauge Teflon® cannula) of the left adrenal vein was performed according to a technique described by Hume and Nelson,¹⁴ which allows selective collection of blood from the adrenal with minimal surgical trauma to surrounding tissues and without disturbing the splanchnic innervation. Heparin was administered at 200 U/kg, iv, followed by 100 U/kg, iv, after 2 h, to avoid clotting of blood in catheters. The total time required for the preparation, from induction, was 1–1½ h.

EXPERIMENTAL DESIGN

Cats were divided into four groups, according to the drug administered prior to the induction of hemorrhage. All drugs were freshly prepared and diluted in 0.9% NaCl. Group I (n = 5) received saline, and group II (n = 5) received the μ -agonist sufentanil (Janssen, R-33800) at a dose of 25 μ g/kg iv, followed by a maintenance infusion. The infusion rate was calculated as:¹⁵

infusion rate (μ g/min)

$$= \text{iv sufentanil bolus} \times (1.4 \text{ t}_{1/2})^{-1},$$

with $t_{1/2}$ being 148 min for iv sufentanil.⁹ Group III (n = 5) received the δ/μ agonist metkephamid (Lilly Research Laboratories, LY 127623 Lot No. P-61553) at a dose of 3 mg/kg iv, and group IV (n = 5) the κ -agonist U50488H (Upjohn Company, Lot No. 0027-RLA-011) at 3.5 mg/kg iv.

Doses were selected on the following basis: 25 μ g/kg iv of sufentanil is considered a very large dose in human and animal studies, providing complete anesthesia.^{9,16} Metkephamid at a dose of 3 mg/kg iv was reported to achieve maximal analgesic effects in rodent experiments.¹⁷ In reference to its long analgesic effects of 4–6 h and the lack of any iv pharmacokinetic data, maintenance infusion was not employed. U50488H at 3.5 mg/kg iv was the highest dose, which was hemodynamically tolerated. In preliminary experiments, the admin-

istration of 7 mg/kg U50488H led to an immediate decline in MABP and acute death of the animal.

In all animals, blood samples were taken simultaneously from the three collection sites: adrenal vein, femoral vein, and femoral artery by syringe pump withdrawal (300 μ l/min) at corresponding time points of the experiment. Baseline samples were taken 1 h after completion of the surgical preparation. The group specific drug (saline, sufentanil, metkephamid, U50488H) was then administered in a bolus of 2 ml over 1 min, and was followed by a continuous infusion of saline (groups I, III, and IV) or sufentanil (group II) at 0.116 ml/min *via* the jugular vein catheter. Two minutes after drug administration, samples S2 were taken. The first phase hemorrhage was then induced by syringe pump withdrawal from the femoral artery at a rate of 2 ml/min. Samples S3 were taken after a blood volume loss of 25%. A period of about 25 min followed until a second hemorrhage was begun at the same rate. Samples S4 were taken immediately after a total blood volume loss of 50%. Blood volume was calculated as 75 ml blood/kg body weight, based on a plasma volume of 47 ml/kg¹⁸ and hematocrit measurements obtained in our laboratory with an average value of 37%. Sample blood volume was included in the overall calculation of total blood volume loss. Animals were killed at the end of the experiment by an overdose of pentobarbital.

METHODS OF SAMPLE PREPARATION AND ASSAYS

Blood samples from the three collection sites (adrenal vein, femoral vein, femoral artery) were drawn simultaneously for about 10 min in EDTA (ethylenediaminetetraacetate disodium) syringes covered by ice. After immediate centrifugation at 4° C for 10 min, plasma for catecholamine assays was pipetted in tubes containing freshly prepared sodium metabisulfate (10 μ l/ml plasma). Plasma for met-enkephalin assays was extracted by Sep-pak separation. All samples were immediately frozen at –70° C until the performance of assays within a week.

Catecholamine assays were performed by high-performance liquid chromatography with electrochemical detection. The assay sensitivity (defined as a 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: 83% for norepinephrine, 81% for epinephrine, 87% for dopamine. Data were corrected for these recoveries. Further details of the assay are described elsewhere.¹⁹

Met-enkephalin was detected by radioimmunoassay with a selective C-terminal directed antibody. The cross reactivity of this antibody was 150% with Met-(O)-enkephalin, 62% with Arg⁶-met-enkephalin, 25% with Phe⁷-Arg⁶-met-enkephalin, <10% with β -endorphin, and <0.04% with metkephamid. The assay sensitivity was 10 pg/tube, the intra-assay variability was 7%, and the interassay variability 12%. The final antibody dilution was 1:800 in a final incubation volume of 0.4 ml/tube. Further details are described elsewhere.²⁰

STATISTICAL ANALYSIS

Hemodynamic and hormonal variables were analyzed in each treatment group. Analyses of hormone levels in adrenal vein, femoral vein, and femoral artery were performed after transformation to logarithms (base 10) to establish homogeneity of variance. In those cases, where baseline values were below the assay sensitivity, the midpoint between zero and assay sensitivity was taken as estimated baseline value. To assess possible changes in the proportional release of catecholamines and met-enkephalin, adrenal vein hormone levels were transformed to molar values, expressed as ratios of epinephrine and transformed to logarithms. A prior one-way analysis of variance (ANOVA) was carried out among groups at individual time points. Within-group changes during the course of the experiment were analyzed by repeated-measures ANOVA. In addition, one-way ANOVA was carried out, to compare adrenal vein, femoral vein, and femoral artery hormone levels in the individual treatment groups at baseline and 50% hemorrhage. If the ANOVA main effects were significant, 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.

Results

At baseline, the four groups of cats were not different in regard to weight, hormone levels, and hemodynamic variables. Table 1 presents hormone levels at baseline, 1 h after surgical preparation during 1 MAC of halothane anesthesia in plasma of 20 cats (groups I, II, III, and IV). Highest levels of all hormones were observed in the adrenal vein. Epinephrine, presenting the highest fraction, exceeded norepinephrine by a factor of 3, dopamine by a factor of 50, and met-enkephalin by a factor of 170. Hormone levels in the femoral vein and femoral artery were similar and significantly lower than in the adrenal vein. Here, norepinephrine presented the main fraction, exceeding epinephrine by a factor of 1.5–2, dopamine by a factor of 3, and met-enkephalin by a factor of 10. Compared to hormone levels in the adrenal vein, systemic levels were only 1/60th for epineph-

TABLE 1. Plasma Levels at Baseline in 20 Cats During 1 MAC Halothane Anesthesia

Sample Site	NE (ng/ml)	EPI (ng/ml)	DA (ng/ml)	ME (pg/ml)
AD	4.17* (3.16, 5.50)	12.50* (8.17, 19.13)	0.23* (0.17, 0.31)	72.44* (57.49, 91.27)
FV	0.39 (0.33, 0.47)	0.26 (0.20, 0.33)	0.10 (0.08, 0.13)	33.88 (28.23, 40.66)
FA	0.29 (0.25, 0.34)	0.15 (0.11, 0.20)	0.10 (0.08, 0.13)	31.62 (27.03, 37.00)

Plasma levels at baseline (geometric means with limits of interval = geometric mean \times \pm geometric SE) for norepinephrine (NE), epinephrine (EPI), dopamine (DA), and met-enkephalin (ME) at three simultaneous collection sites: adrenal vein (AD), femoral vein (FV), and femoral artery (FA). Data from 20 cats with the following hemodynamic variables (mean \pm SE): mean arterial blood pressure: 125 \pm 5 mmHg; pulse pressure: 30 \pm 2 mmHg; heart rate: 212 \pm 6 bpm.

* $P < 0.05$ compared to FV and FA values.

rine, 1/20th for norepinephrine, and 1/2 for dopamine and met-enkephalin.

EFFECTS OF DRUG ADMINISTRATION ON BASELINE CONDITIONS

The effects of the different drugs, saline, sufentanil, metkephamid, and U50488H on hemodynamic variables during baseline conditions are shown in representative on-line tracings of four individual cats (fig. 1). Heart rate, mean arterial blood pressure (MABP), and pulse pressure in the four different treatment groups at distinct time points of the experiment are presented in figure 2. There were no statistically significant differences in the baseline hemodynamic values of the four groups. The administration of saline did not evoke any changes. The administration of sufentanil (25 μ g/kg iv) produced an immediate brief decrease in blood pressure and heart rate (fig. 1), followed by a marked and persistent elevation in blood pressure within 2 min after the start of the injection. The rise in blood pressure was accompanied by a more than doubling in pulse pressure, representing significantly higher values than in the saline group (fig. 2). Concomitantly, maximal pupillary dilatation, which persisted throughout the experiment, was observed in all animals.

Administration of metkephamid (3 mg/kg iv) led to an immediate decrease in blood pressure, and evoked an immediate increase in pupillary size from pre-drug slit form to approximately half maximal dilatation.

In U50488H (3.5 mg/kg iv)-treated animals, a significant and prolonged decrease in MABP, as compared to baseline and saline controls, was consistently observed, and no changes in pupil size occurred.

Hormonal responses to drug administration in adrenal vein, femoral vein, and femoral artery are shown in figures 3–5. Administration of saline and U50488H did

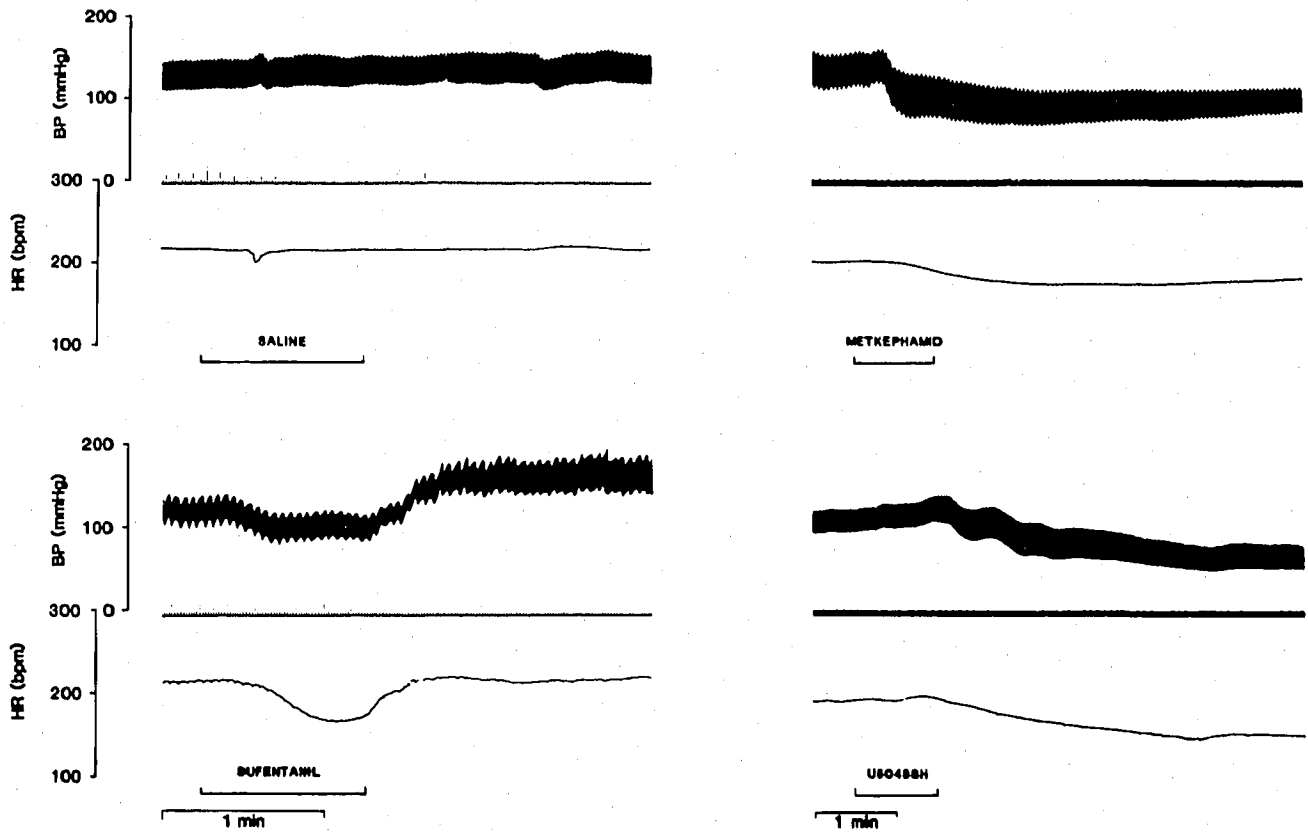


FIG. 1. Effects of the administration of saline, sufentanil (25 μ g/kg iv), metkephamid (3 mg/kg iv), and U50488H (3.5 mg/kg iv) on BP and HR response in four representative cats. Drugs were administered in a volume of 2 ml over a time period of 1 min.

not evoke any changes in adrenal or systemic hormone levels. In contrast, sufentanil alone, prior to hemorrhage, evoked a significant ten- to 20-fold increase in adrenal vein catecholamine and a six-fold increase in adrenal vein met-enkephalin levels. Concurrently, a significant rise in norepinephrine and epinephrine levels was observed in the femoral vein (six-fold) and in the femoral artery (20-fold for epinephrine, six-fold for norepinephrine). Metkephamid did not markedly influence adrenal medullary secretion. However, a significant four- to five-fold increase in systemic met-enkephalin levels was observed after drug administration.

EFFECTS OF STAGED HEMORRHAGE

Twenty-five Percent Hemorrhage. Hemodynamic changes during staged hemorrhage are presented as part of figure 2, and hormonal changes in adrenal vein, femoral vein, and femoral artery as part of figures 3–5. With a slow hemorrhage of 25% of the total blood volume, a significant drop in MABP (-56 mmHg) compared to post-drug values was observed in cats receiving saline. The pronounced fall in MABP did not evoke a significant increase in adrenal medullary secretion.

However, a three-fold increase in epinephrine levels in the femoral artery at this time reached significance. Cats receiving sufentanil differed from animals receiving saline in so far, as during the 25% hemorrhage, no significant drop in MABP occurred, and, thus, significantly higher levels of MABP (120 mmHg versus 78 mmHg) were maintained. This presumably reflects the initial stimulation effect observed with sufentanil under pre-hemorrhage conditions. No major hemodynamic or hormonal changes compared to post-drug values occurred in cats treated with metkephamid and U50488H, and no difference was observed in comparison to the saline group. Only systemic met-enkephalin levels in the metkephamid group which were elevated after drug administration showed a decrease back to baseline levels under 25% hemorrhage.

Fifty Percent Hemorrhage. In cats receiving saline, hemorrhage of 50% of total blood volume resulted in no further fall in MABP (-70 mmHg from post-drug value) compared to the first stage 25% hemorrhage. However, at this stage, with a MABP of 65 mmHg, a significant increase in adrenal catecholamine levels (30-fold for norepinephrine, 14-fold for dopamine, and ten-fold for epinephrine) was observed compared to

HEMODYNAMIC VARIABLES

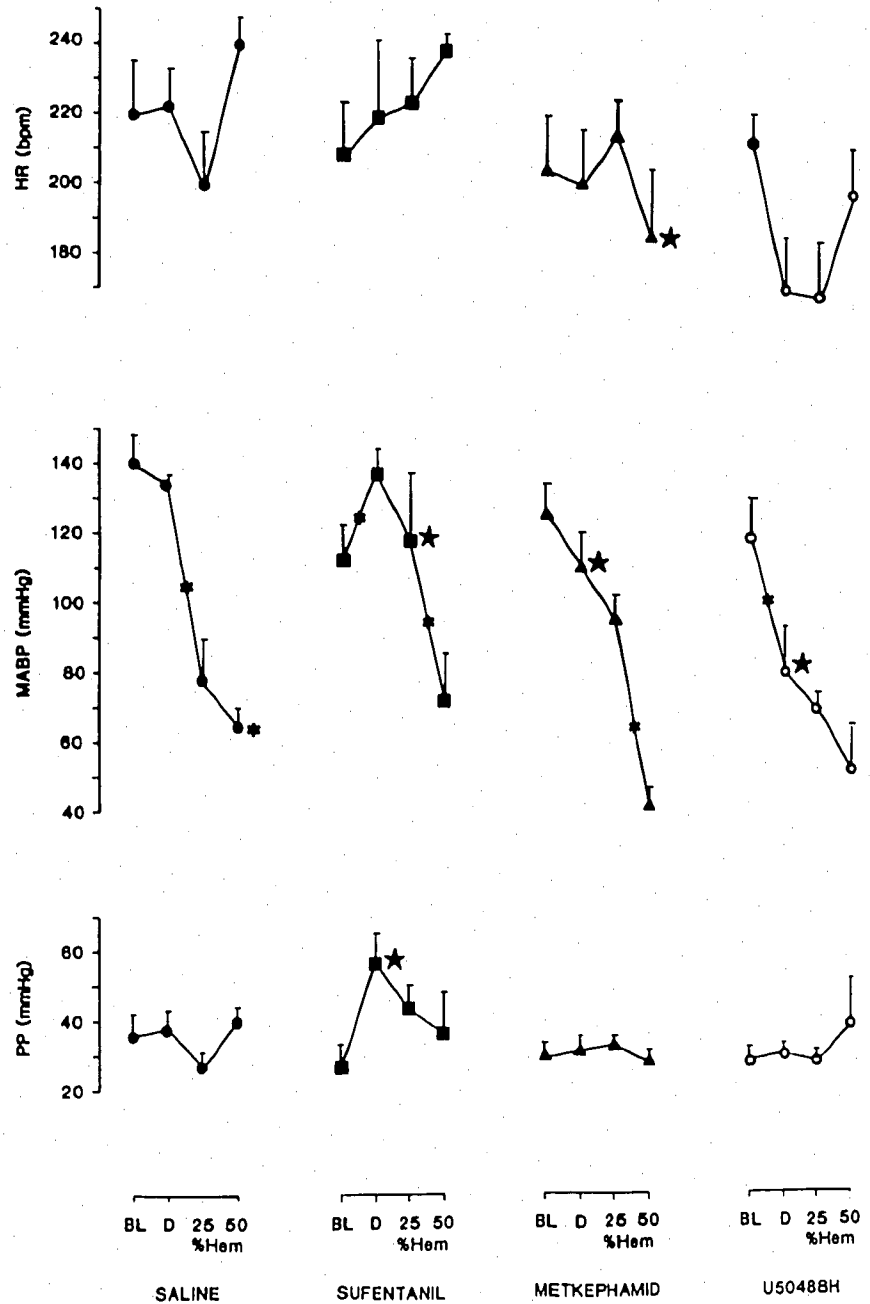


FIG. 2. Hemodynamic variables: heart rate (HR), mean arterial blood pressure (MABP), and pulse pressure (PP) (mean \pm SE) in four groups of cats ($n = 5$ in each group) at baseline (BL), after drug (D) administration (saline, sufentanil, metkephamid, U50488H), and after 25% and 50% hemorrhage (Hem). Stars denote significant differences in comparison to the saline group. Asterisks refer to within-group comparisons and refer to pre-drug (BL vs. D) and post-drug (D vs. 25% and 50% Hem) comparisons. Asterisks on lines connecting 25% and 50% Hem mark significant differences between these points, as well as between 50% Hem and post-drug (D) value. Asterisks behind the 50% Hem point mark a significant difference compared to post-drug (D) value, but not to 25% Hem.

post-drug values, whereas met-enkephalin levels did not increase significantly (five-fold). Systemic levels paralleled the changes observed in the adrenal vein with a 15- to 30-fold rise of norepinephrine and epinephrine levels in femoral vein and femoral artery, and an eight-fold rise of dopamine levels in the femoral vein. Cats receiving sufentanil showed a significant decrease in MABP and a significant rise in adrenal vein dopamine (25-fold) and norepinephrine (14-fold) levels compared

to post-drug values. Epinephrine and met-enkephalin levels at 50% hemorrhage were significantly increased (four-fold) compared to values at 25% hemorrhage. Concurrently measured systemic hormone levels showed a five- and three-fold increase of norepinephrine in femoral vein and femoral artery, respectively. Cats treated with metkephamid showed a pronounced fall in MABP to 42 mmHg, accompanied by a decrease in heart rate to 186 bpm, which represented a signifi-

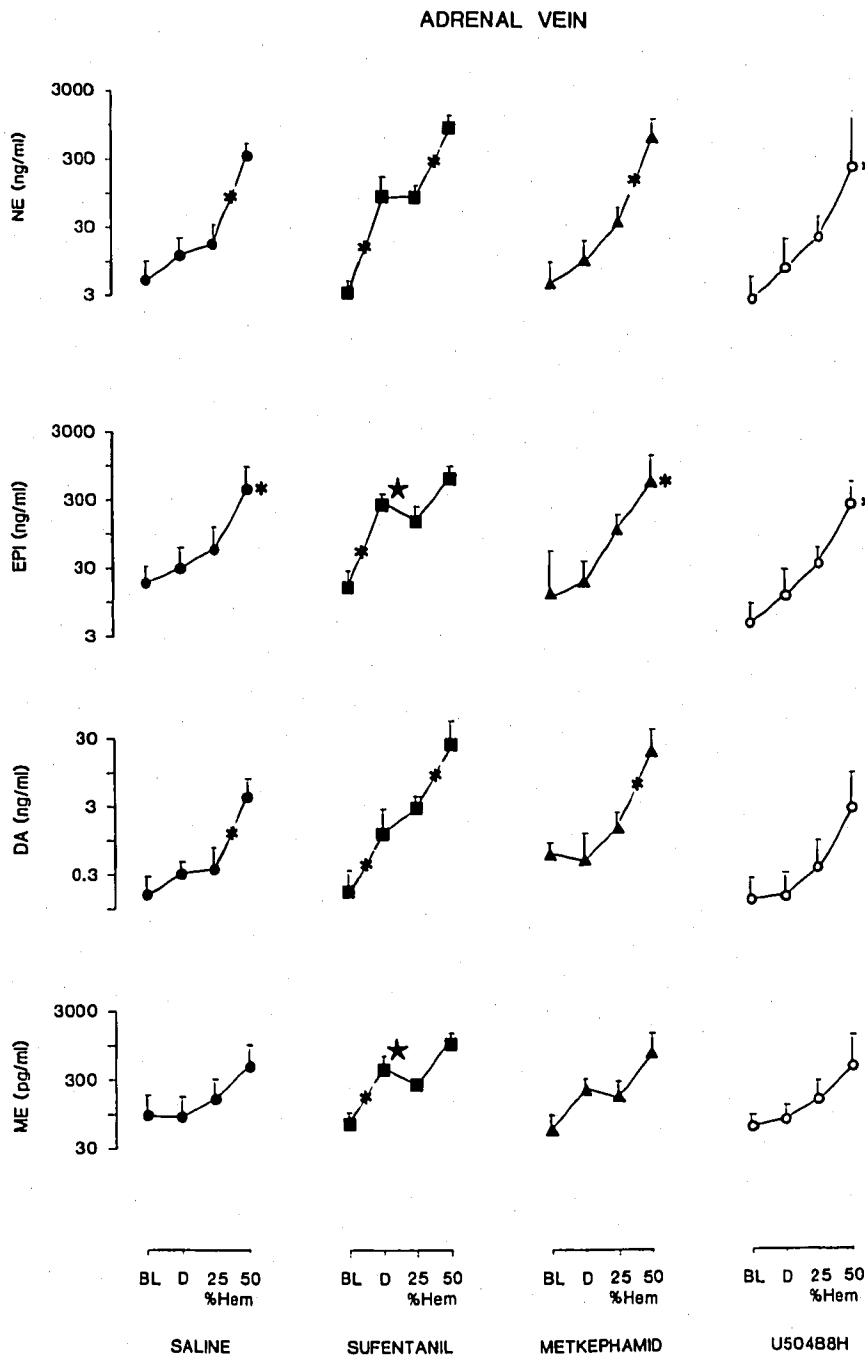


FIG. 3. Adrenal vein plasma levels for norepinephrine (NE), epinephrine (EPI), dopamine (DA), and met-enkephalin (ME) (geometric mean \pm geometric SE) in four groups of cats ($n = 5$ in each group) at baseline (BL), after drug (D) administration (saline, sufentanil, metkephamid, U50488H), and after 25% and 50% hemorrhage (Hem). Stars denote significant differences in comparison to the saline group. Asterisks refer to within-group comparisons and refer to pre-drug (BL vs. D) and post-drug (D vs. 25% and 50% Hem) comparisons. Asterisks on lines connecting 25% and 50% Hem mark significant differences between these points, as well as between 50% Hem and post-drug (D) value. Asterisks behind the 50% Hem point mark a significant difference compared to post-drug (D) value, but not to 25% Hem. In the sufentanil group, though not marked specifically, EPI and ME values at 50% hemorrhage were significantly different from values at 25% Hem.

cantly lower value than observed in cats which received saline (241 bpm). Adrenal vein catecholamine levels were significantly elevated: 70-fold for norepinephrine, 37-fold for dopamine, and 30-fold for epinephrine, compared to post-drug values. This increase was paralleled by a ten- to 25-fold rise in systemic hormone levels, with the exception of dopamine in the femoral artery, which did not change. In cats receiving U50488H, no further fall in MABP (53 mmHg) was

noted during the 50% hemorrhage, compared to post-drug values (81 mmHg). However, also in this group, adrenal vein hormone levels showed a significant rise in norepinephrine (35-fold) and epinephrine (23-fold) compared to post-drug values. Concurrently, ten- to 20-fold increases of systemic norepinephrine and epinephrine levels were observed.

Overall, after 50% hemorrhage, there were no differences with regard to adrenal vein and systemic hor-

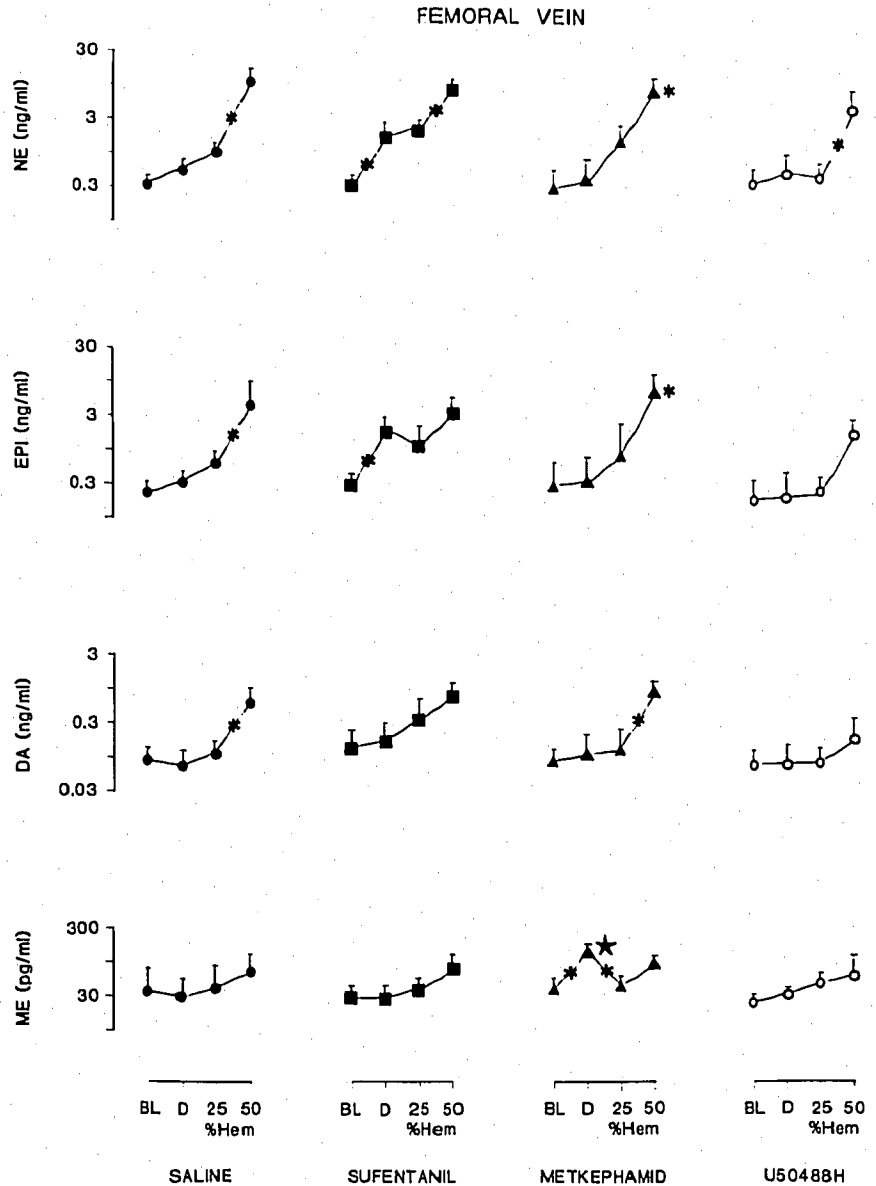


FIG. 4. Femoral vein plasma levels for norepinephrine (NE), epinephrine (EPI), dopamine (DA), and met-enkephalin (ME), corresponding to adrenal vein plasma levels (fig. 3). Abbreviations and statistical analyses are the same as described in figure 3. In the metkephamid group, though not marked specifically, the ME value at 50% Hem was significantly different from the value at 25% Hem.

more levels in cats receiving μ -, δ -, or κ -agonists, compared to cats receiving saline. Generally, throughout the experiment, hormone levels in femoral vein and femoral artery were similar and consistently lower than in the adrenal vein. After 50% hemorrhage, hormone levels in the adrenal vein exceeded femoral vein and femoral artery levels significantly, roughly by a factor of 100 for norepinephrine and epinephrine, by a factor of 20 for dopamine, and by a factor of 8 for met-enkephalin. As previously shown in similarly conducted cat experiments, there is only a poor correlation between adrenal vein and systemic levels of catecholamines and met-enkephalin.[†]

[†] Gaumann D, Yaksh T: Unpublished observations.

MOLAR RATIOS

The molar ratios of adrenal vein hormone levels in comparison to epinephrine were calculated at different time points of the experiment (table 2) to evaluate the proportional release of catecholamines and met-enkephalin after drug administration and during staged hemorrhage in the different treatment groups. No differences between groups were observed at baseline and throughout the experiment. However, administration of sufentanil led to a significant decrease in met-enkephalin/epinephrine ratios, compared to group baseline values. After 50% hemorrhage, a significant increase in norepinephrine/epinephrine ratios, as compared to post-drug ratios, was observed in saline- and sufentanil-treated cats.

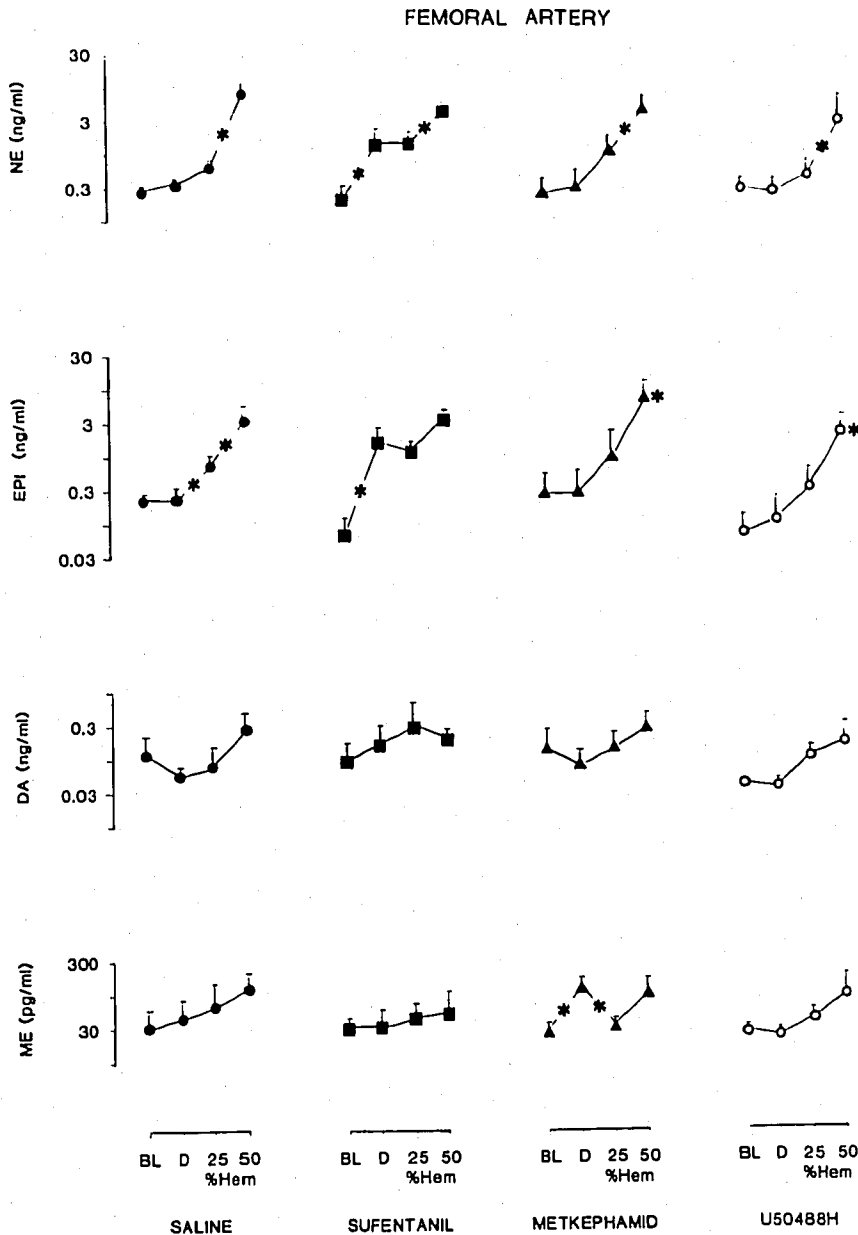


FIG. 5. Femoral artery plasma levels for norepinephrine (NE), epinephrine (EPI), dopamine (DA), and met-enkephalin (ME), corresponding to adrenal vein (fig. 3) and femoral vein (fig. 4) plasma levels. Abbreviations and statistical analyses are the same as described in figure 3. In the metkephamid group, though not marked specifically, the ME value at 50% Hem was significantly different from the value at 25% Hem.

Discussion

In the halothane (1 MAC)-anesthetized cat, induced hypovolemia results in a volume-dependent fall in blood pressure. This response is accompanied by a baroreceptor-mediated stimulation of sympathetic outflow which evokes the secretion of catecholamines and met-enkephalin from the adrenal. As will be discussed below, ample evidence suggests that adrenal secretion may be altered either directly or indirectly by the activation of opioid receptors. In this light, the most striking finding in the present experiments is the fact that μ -, δ -, or κ -receptor agonists at pharmacologically significant

doses do not alter the adrenal secretory response to hemorrhage. The possibility that yet higher dosages of the opioid agonists might have yielded modulatory and/or suppressive effects of the adrenal secretory response has to be considered. Doses in the present studies were chosen on the basis of side effects (sufentanil) or morbidity (U50488H). The metkephamid dose was sufficient to produce a mild, but significant, fall in blood pressure. Thus, while it is possible that higher doses might have resulted in an effect on hypovolemia-stimulated secretion, the present studies showing no effect with doses which were pharmacologically active argue against this interpretation. Moreover, current studies in

our laboratory examining the effects of splanchnic nerve stimulation (5 and 50 Hz) on the adrenal secretory response in cats during 1 MAC of halothane anesthesia indicate that even dosages of sufentanil as high as 250 $\mu\text{g}/\text{kg}$ iv do not suppress the adrenal release of catecholamines and met-enkephalin.¹¹ Thus, at present, we conclude that certainly exogenously administered μ -preferring opioids do not act either on the cholinergic link between the splanchnic nerve and the adrenal, or on the adrenal chromaffin cells themselves, to alter detectably their secretion. The results in the present experiments also correspond to recently conducted experiments in an identical cat preparation, in which the administration of the opioid antagonist, naltrexone (in a range of dosages 0.01 to 10 mg/kg iv) prior to the induction of 25% and 50% hemorrhage had no effect on adrenal secretory and hemodynamic responses.¹¹ This excludes a prominent effect of endogenous opioids in the sympathetic response evoked by hemorrhage in this model.

Stimulatory effects on adrenal hormone secretion and hemodynamics, accompanied by pupillary dilatation, following the administration of sufentanil point to a central action of this drug. In contrast to humans, morphine and morphine-like drugs have been described to evoke excitation in unanesthetized cats.²¹ Also, in other species, increased spontaneous sympathetic outflow has been observed following microinjections of μ -agonists into the third ventricle,²² the anterior hypothalamus,²³ the cisterna magna, the hypothalamic paraventricular nucleus, and the brainstem nucleus of the solitary tract.²⁴ In recent experiments under similar conditions (1 MAC halothane anesthesia), cats which were acutely transected at the T₃₋₄ level showed no changes in adrenal vein catecholamine and met-enkephalin levels following the administration of sufentanil (25 mg/kg iv).¹¹ This further emphasizes the central stimulating action of sufentanil, as well as the lack of direct actions on the adrenal medulla. However, in these animals, sufentanil continued to evoke a significant increase in MABP. We presume that, due to this central stimulation, the MABP was significantly elevated in sufentanil-treated cats during the 25% hemorrhage compared to cats receiving saline. This observation corresponds to rat experiments conducted by Feuerstein.²⁵ In these studies, after approximately 25% hemorrhage, the administration of a μ agonist (DAGO) into the anterior hypothalamus enhanced the recuperation of blood pressure and stimulated the heart rate response.

Metkephamid, a mixed δ and μ receptor agonist, was devoid of significant effects on the adrenal hormone secretion, but evoked a significant increase in met-enkephalin levels measured in femoral artery and vein

TABLE 2. Molar Ratios of Adrenal Vein Hormones Expressed as Fraction of Epinephrine during Time Course of Experiment

	NE/EPI	DA/EPI	ME/EPI · 10 ⁻³
Baseline	0.36 (0.28, 0.46)	0.02 (0.01, 0.03)	0.62 (0.42, 0.92)
Drug			
Saline	0.44 (0.38, 0.51)	0.01 (0.01, 0.01)	0.91 (0.29, 2.81)
Sufentanil	0.32 (0.20, 0.52)	0.01 (0.01, 0.02)	0.64 (0.55, 0.75)*
Metkephamid	0.51 (0.34, 0.77)	0.03 (0.02, 0.06)	0.29 (0.16, 0.54)
U50488H	0.72 (0.56, 0.93)	0.01 (0.00, 0.02)	1.66 (0.71, 3.88)
25% Hemorrhage			
Saline	0.33 (0.26, 0.42)	0.01 (0.00, 0.02)	0.87 (0.28, 2.69)
Sufentanil	0.65 (0.41, 1.03)	0.02 (0.01, 0.03)	0.35 (0.27, 0.45)
Metkephamid	0.35 (0.30, 0.41)	0.02 (0.02, 0.03)	0.48 (0.40, 0.58)
U50488H	1.48 (1.07, 2.04)	0.01 (0.00, 0.02)	0.98 (0.80, 1.21)
50% Hemorrhage			
Saline	1.26 (0.91, 1.74)†	0.02 (0.02, 0.02)	0.47 (0.21, 1.03)
Sufentanil	1.32 (1.13, 1.54)†	0.04 (0.03, 0.06)	0.48 (0.39, 0.59)
Metkephamid	1.15 (0.62, 2.14)	0.04 (0.02, 0.07)	0.34 (0.28, 0.42)
U50488H	1.07 (0.59, 1.95)	0.01 (0.01, 0.02)	0.51 (0.34, 0.77)

Molar ratios (geometric means with limits of interval = geometric mean \times geometric SE) of adrenal vein plasma levels for norepinephrine (NE), dopamine (DA), and met-enkephalin (ME) expressed as fractions of epinephrine (EPI) in 20 cats at baseline, after drug administration, and after induction of 25% and 50% hemorrhage. Administered drugs were saline, the μ -agonist sufentanil (25 $\mu\text{g}/\text{kg}$ iv followed by maintenance infusion), the δ/μ agonist metkephamid (3 mg/kg iv), and the κ -agonist U50488H (3.5 mg/kg iv) (n = 5 in each treatment group). Since no differences were noted between groups at baseline, data were pooled for presentation (* $P < 0.05$ compared to group baseline ratio; † $P < 0.05$ compared to group post-drug ratio).

plasma. This observation, in light of the failure of sufentanil to have an equivalent effect, might be interpreted to indicate a δ -receptor mediated effect on opioid release mechanisms. This selective increase in met-enkephalin plasma levels in femoral artery and vein following metkephamid may be explained by the release of met-enkephalin from other autonomic ganglia or the gastrointestinal tract, where met-enkephalin is also found in abundance.²⁶ Marked hemodynamic suppressant effects following the administration of metkephamid and, more so, U50488H may be explained by central and/or peripheral actions of these drugs. A decrease in heart rate and blood pressure has been shown following microinjections of δ - and κ -receptor agonists in the nucleus periventricularis hypothalami,²⁷ and of κ -receptor agonists into the nucleus ambiguus and the nucleus tractus solitarius of the rat.²⁸ Hypotension and

bradycardia evoked by the peripheral activation of pulmonary J-receptors has been described following the intravenous administration of δ - and μ -agonists.²⁹ Further, the observed decrease in blood pressure may have been also mediated by a direct action of δ - and κ -agonists on arterial prejunctional opioid receptors, thus reducing the release of norepinephrine and inhibiting vasoconstriction.³⁰ Direct depressing effects of metkephamid and U50488H on the heart *via* specific δ and κ receptors may also explain the decrease in heart rate and blood pressure. In isolated rat atria, enkephalins have been shown to attenuate the positive chronotropic effects of norepinephrine,³¹ and the κ -agonist dynorphin was shown to significantly reduce the contraction amplitude of guinea pig atria.³²

Finally, we face the issue, why do opioids, known to be powerful suppressants of the secretory adrenal medullary response evoked by pain, not suppress the adrenal medullary hormone secretion evoked by hypovolemia? The present findings, suggesting the non-involvement of opioids in the baroreceptor-mediated sympatho-adrenal stimulation, correspond to clinical observations during anesthesia with high-dose μ -receptor agonists for cardiac surgery. As widely appreciated, the adrenal catecholamine secretion during cardiopulmonary bypass is not suppressed by even excessive doses of opioids.³³ The mechanism of this apparent selectivity is not known. Excitatory drive into preganglionic neurons from baroreceptors arises from bulbospinal projections (see Loewy³⁴). Similarly, ample evidence suggests that, in the intact animal, afferent somatic input which excites sympathetic outflow acts mainly *via* a spinal-bulbar-spinal loop.³⁵ We are not aware of any evidence as to whether the bulbospinal pathways activated by the somatic and baroreceptor stimuli are the same or different. Based on the present studies, two speculative considerations may be presented. First, the functional selectivity may simply result because opioid receptors are only associated with small somatic afferents and/or the processing elements of the pain pathway, and not with the baroreceptor systems which are processed through the brainstem. Alternatively, opioid binding and enkephalin containing terminals have been identified in the intermediolateral cell column,^{36,37} and, as previously discussed, there are opioid receptors associated with the adrenal chromaffin cells and the presynaptic inhibition of acetylcholine release from sympathetic ganglia.^{4-7,38} Thus, one would, in fact, anticipate a direct effect by opiates on intermediolateral cell column activity evoked by bulbospinal excitation secondary to both baroreceptor and somatic stimuli. This does not appear to be the case. Opioid receptors can selectively modulate postsynaptic activity. Thus, for example, in dorsal horn neurons receiving converging input

from several classes of afferents, opiates may suppress activity evoked by small-, but not large-, diameter afferents.^{39,40} This selectivity may derive from the ability of opiates to effectively shunt membrane excitatory postsynaptic potentials evoked by slowly, but not rapidly, conducting fibers (see⁴¹). Alternatively, the excitatory effects of input onto cells in the intermediolateral cell column may differ significantly. Thus, following high-intensity sciatic nerve stimulation in a comparable cat model, epinephrine levels in the adrenal vein rise by a factor of 3-5;⁴² whereas, during hypovolemia, these levels rise by a factor of approximately 30-50. We might, thus, speculate that baroreceptor-activated drive may simply represent a more effectively coupled stimulus for adrenal activation than even a strong somatic stimulus. Whether these relationships apply to preganglionic cells in the intermediolateral cell column is not known; but, if so, it would indicate that the physiological characteristics of the excitatory drive onto the preganglionic sympathetic neurons evoked by somatic pain differs from that evoked by baroreceptor stimulation.

From a teleological standpoint, regarding survival chances of the individual, one might speculate that centrally acting opioids exerting beneficial effects in suppressing unnecessary pain sensations together with the accompanying endocrine response do not interfere, and thus preserve, the existentially important sympatho-adrenal reflex evoked by hypovolemia.

We conclude that systems processing the adrenal response to cardiovascular reflexes can be distinguished pharmacologically from those systems mediating the autonomic response evoked by pain.

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