Antinociceptive and cardiovascular properties of esmolol following formalin injection in rats

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Purpose: To assess the role of esmolol, a $ß_1$ receptor blocker, in the modulation of pain in the absence of anesthesia.

Methods: Rats were chronically instrumented to record mean arterial blood pressure (MAP) and heart rate (HR). Animals were divided into three groups. Group 1 [esmolol high (EH) 150 $mg \cdot kg^{-1} \cdot hr^{-1}$; n = 9], Group 2 [esmolol low (EL) 40 $mg \cdot kg^{-1} \cdot hr^{-1}$; n = 7] and Group 3 saline (n = 9). Formalin 5% was injected in the rat hind paw. Formalin-induced lifting, MAP and HR were recorded at five minute intervals for 35 min after formalin injection.

Results: Formalin was associated with an early (Phase 1; 0-5 min) and late nociceptive response (Phase 2; 10-35 min). Esmolol did not affect Phase 1. Although low dose esmolol had minimum effects on nociceptive Phase 2, it was diminished with high dose esmolol. Formalin induced biphasic increases in MAP and HR. Although esmolol did not affect the initial increase in MAP, high dose esmolol blunted the secondary increase in MAP. Both low and high doses of esmolol inhibited formalin-induced tachycardia during the first 30 min.

Conclusion: Our data suggest that esmolol leads to analgesia and reduction of cardiovascular responses to pain.

Objectif : Cette étude a été réalisée pour étudier le rôle de l'esmolol, un bloqueur des récepteurs béta 1 dans la modulation de la douleur en l'absence d'anesthésie.

Méthode : Des rats ont été instrumentés pour enregistrer la tension artérielle moyenne (TAM), et la fréquence cardiaque (FC). Les animaux ont été divisés en 3 groupes. Le groupe 1 [esmolol à la dose de 150 $\mu g \cdot kg^{-1} \cdot hr^{-1}$; n = 9], le groupe 2 [esmolol à la dose de 40 $\mu g \cdot kg^{-1} \cdot hr^{-1}$; n = 7] et le groupe 3 [chlorure de sodium isotonique; n = 9). Le formol (5 %) a été injecté par voie sous-cutanée dans la patte du rat. Les mouvements de la patte ainsi que la TAM et la FC ont été enregistrés à 5 min d'intervalles pendant 35 min après l'injection du formol.

Résultats : L'activité physique qui accompagnait l'administration de formol a été représentée par une phase immédiate (Phase 1; 0-5 min) et une phase secondaire (Phase 2; 10-35 min). L'esmolol n'a pas produit d'effets significatifs sur les mouvements de la patte en Phase 1. Alors qu'à faible dose, l'esmolol n'a provoqué que peu d'effets sur les mouvements de la patte en Phase 2, à forte dose il a engendré une réponse significative. Le formol a produit des augmentations significatives de la TAM et de la FC. Bien que l'esmolol, à forte dose, n'a pas produit de changements significatifs sur la TAM, il a fortement diminué l'augmentation secondaire de la TAM. Les 2 doses d'esmolol ont diminué la tachycardie provoquée par l'injection de formol.

Conclusion : Nos résultats suggèrent que l'administration d'esmolol s'associe à une diminution de l'hyperactivité liée à l'injection de formol dans la patte.

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ESMOLOL has been postulated to reduce anesthetic requirements via a direct antinociceptive property.[1] Although involvement of the sympathetic system in nociception is well established, mechanisms linking the sensory and sympathetic nervous systems are unclear.[2] The sympathetic nervous system is involved in pain via the potentiation of the release of mediators such as interleukin-8[3] and increased sensitization to substance P.[4] It is also well established that $_2$ adrenergic agonist receptors stimulated by agonists in the spinal cord[5] and the locus caeruleus in the medulla plays a major role in the inhibition of nociception.[6] Pain models differ in their responses to sympathetic blockade. Experimentally, several models of pain have been developed. These models explore different pathways and potentially different mechanisms, especially with respect to the role played by the sympathetic nervous system. Thus, guanethidine, a sympatholytic agent, has analgesic properties in chronic neuropathie, but not in chronic arthritic pain.[7] Models exploring acute pain pathways also exist, among them the conscious rat subjected to the formalin test.[8–10] The formalin test model produces a biphasic response: the early nociceptive response (0-5 min), possibly related to a C fibre activation and the late nociceptive response (10-35 min) that appears to be dependent on a combination of inflammatory reactions in the peripheral tissue and facilitation of spinal transmission.[11–12]

Although the analgesic properties of alpha agonists in surgical patients have been extensively documented,[13] the role played by esmolol in the modulation of postoperative pain remains to be established. Interestingly, Cunha *et al.* (1991) reported that atenolol and propranolol inhibited IL-8-evoked hyperalgesia. Esmolol, which is primarily indicated in the treatment of hypertension and tachycardia during anesthesia, has been claimed also to modulate pain.[3] Clinical evidence obtained during anesthesia with esmolol, suggests that esmolol may modulate the pain pathways. Evidence supporting this concept includes: 1) Esmolol has similar properties to alfentanil as a supplement to propofol / N_2O anesthesia;[14] 2) Esmolol has anesthetic sparing effects, reducing the anesthetic requirements for skin incision during propofol / N_2O and morphine anesthesia in humans;[1] 3) Esmolol prevents the cardiovascular and neuroendocrine response to electroconvulsive therapy.[15] However, direct evidence supporting the concept that esmolol modulates pain pathways remains to be established.

This study was designed to assess the role of esmolol in the modulation of acute pain and the associated cardiovascular changes in conscious, chronically instrumented rats subjected to the formalin test.

Materials and methods

Cardiovascular instrumentation

After approval from the Animal Welfare Committee of the University of Texas Medical School, Sprague Dawley rats (250–350 gr) were anesthetized with halothane 2%, tracheas intubated and lungs ventilated under isothermic conditions. Tygon PE 50 catheters (Tygon, Cole-Palmer Instrument Co., Chicago, IL) were introduced into the abdominal aorta via the femoral artery to record arterial blood pressure and heart rate and into the femoral vein for drug administration, respectively. Catheters were tunneled to the dorsum of the neck for externalization and the surgical wounds were closed. Intraoperative care was facilitated by the infiltration of bupivacaine 0.25% into the wounds. Animals recovered from surgery for at least five days before initiation of the experimental protocol. Buprenorphine, 0.01-0.02 $mg \cdot kg^{-1}$, was administered on one occasion after completion of the surgery. Antibiotic therapy (5 $mg \cdot kg^{-1}$ gentamycin) was initiated for five days postoperatively. To avoid damage to the implanted catheters, animals were housed in individual cages in an air-conditioned, light controlled room (12 hr light, 12 hr dark) and were allowed to mobilize freely.

Experimental design

Twenty-five rats were divided into three groups. Group 1 [esmolol high (EH); n=9] received an *iv* bolus of esmolol (600 $mg \cdot kg^{-1}$) followed by a continuous *iv* infusion of 150 $mg \cdot kg^{-1} \cdot hr^{-1}$ throughout the study period. Group 2 [esmolol low (EL); n=7] received an *iv* bolus of esmolol (150 $mg \cdot kg^{-1}$) followed by an infusion of 40 $mg \cdot kg^{-1} \cdot hr^{-1}$. Animals of Group 3 (n= 9) received saline, the solvent of esmolol, as a bolus followed by a continuous infusion at volumes equal to those administered in previous groups and served as the control. The volumes of the bolus as well as the volume of the infusion were normalized to 1 ml and 2 $ml \cdot hr^{-1}$, respectively in all groups.

Experiments were initiated five days following experimental surgery. Animals of all groups were placed on a metal mesh screen (20 cm x 20 cm). Systolic and diastolic arterial blood pressures were recorded with a P50 Statham pressure transducer (Gould, Cleveland, OH) connected to the PE-50 arterial catheter. Mean arterial blood pressure was electronically derived and simultaneously displayed. Heart rate was continuously recorded with a Gould tachometer (Gould, Cleveland, OH) that was triggered by a differential arterial pressure signal. The femoral vein was connected to a syringe driver (Medfusion, Medex, Inc., Duluth, GA) from the PE-

50 tubing and used for drug administration. Mean arterial pressure and heart rate were continuously recorded at least 30 min following acclimation and before the initiation of the experiment, and for 35 min following the formalin injection.

When steady state was achieved following esmolol (20 min) or saline infusion (20 min), 30 ml formalin 5% (30 ml) were injected to awake rats. Injections were performed at the plantar surface of the rat hind paw with a 28 gauge needle attached to a 50 ml Hamilton syringe with PE-10 tubing.

Mean arterial pressure and heart rate were collected at five minutes into intervals for 35 min following the formalin injection. Formalin-induced nociceptive behaviour was assessed by a blinded observer. To quantify the formalin responses, the instances of spontaneous lifting were counted at 0-5 and at five minute intervals during 10-35 min after formalin injection. Formalin injected into the rat hind paw induced a biphasic lifting behaviour. An initial acute phase (Phase 1: during the 0-5 min after the formalin injection followed by a prolonged tonic response (Phase 2: beginning about 10 min after the formalin injection). For data analysis, Phase 1 and Phase 2 were examined separately. Observations were carried out for 35 min after formalin injection.

Statistical analysis

Hemodynamic and nociceptive changes among groups were analyzed by one way analysis of variance (ANOVA). When significant, an appropriate multiple comparison method (Dunnett's t test) was applied.[16] All values are presented as mean ± SEM. P <0.05 was considered significant.

Results

No behavioural changes occurred following either esmolol or saline treatments. Following completion of the study, all rats had normal stepping and righting reflexes.

Nociceptive scoring: Figures 1 and 2 represent paw lifting time and cumulative instances of Phase 1 and Phase 2, respectively following formalin injection in rats treated with saline, low and high doses of esmolol. Subcutaneous injection of formalin resulted in two-phase nociceptive behaviour in all groups. The biphasic nociceptive behaviours as assessed by lifting following the formalin test has been extensively described.[8–10] The primary phase (Phase 1) response was immediate and lasted up to five minutes following formalin injection. Esmolol did not affect the Phase 1 response. Although the low dose of esmolol had minimum effects on the secondary and longer lasting response (Phase 2), the nociceptive behaviours were diminished with high dose of esmolol. An increase in lifting, but of a lesser magnitude compared with saline, occurred only at 30 and 35 min.

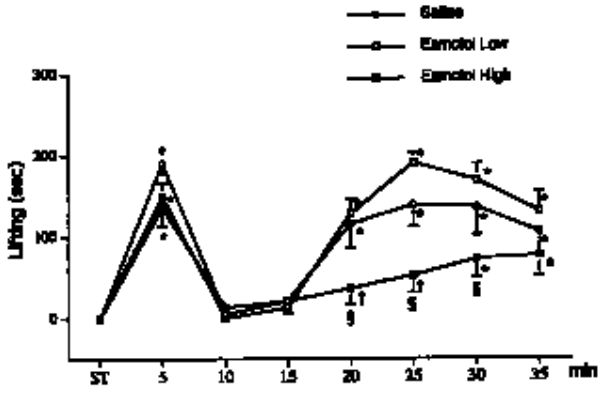

FIGURE 1 Paw lifting time following saline (n=9), esmolol low (n=7) and esmolol high (n=9) in rats subjected to formalin injection. Data are presented in actual changes from ST (mean ± SEM); ST = steady state
**P* < 0.05 *vs* ST. †*P* < 0.05 *vs* saline. ‡*P* < 0.05 *vs* esmolol low

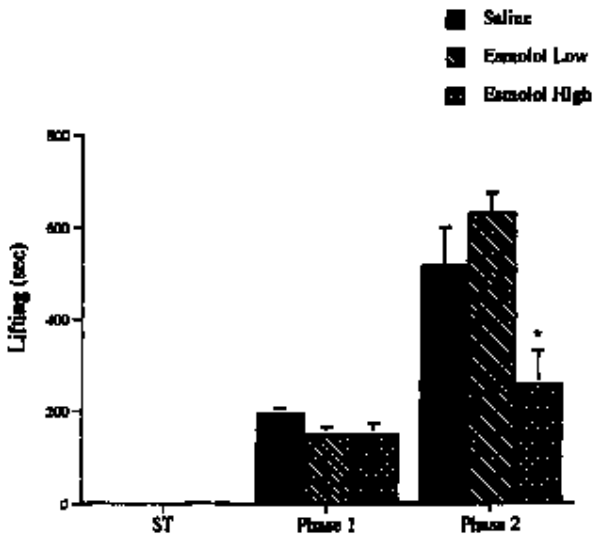

FIGURE 2 Cumulative paw lifting time in (early, 0-5 min) Phase 1 and paw lifting time in (late, 10-35 min) Phase 2 following saline (n=9), esmolol low (n=7) and esmolol high (n=9) in rats subjected to formalin injection; ST = steady state
**P* < 0.05 *vs* saline

Cardiovascular measurements: The Table represents mean arterial pressure and heart rate before and

TABLE Effects of saline (n=9), esmolol high (150 μg·kg^{-1}·hr^{-1}; n= 9) and esmolol low (40 μg·kg^{-1}·hr^{-1}; n=7) administered intravenously on mean arterial pressure (MAP) and heart rate (HR) prior to drug administration (baseline) and at steady state (before formalin injection). Data are represented as mean ± SEM. *$P < 0.05$ *vs* baseline.

		Baseline	*Steady State*
Saline	MAP (mmHg)	121.9 ± 2.7	119.5 ± 1.8
	HR (bt·min^{-1})	424.4 ± 11.2	421.9 ± 9.6
Esmolol High	MAP (bt·min^{-1})	116.5 ± 2.1	103.7 ± 1.8*
	HR (bt·min^{-1})	423.0 ± 7.6	359.0 ± 6.0*
Esmolol Low	MAP (bt·min^{-1})	122.7 ± 3.7	112.1 ± 4.0*
	HR (bt·min^{-1})	435.7 ± 13.6	380.0 ± 8.7*

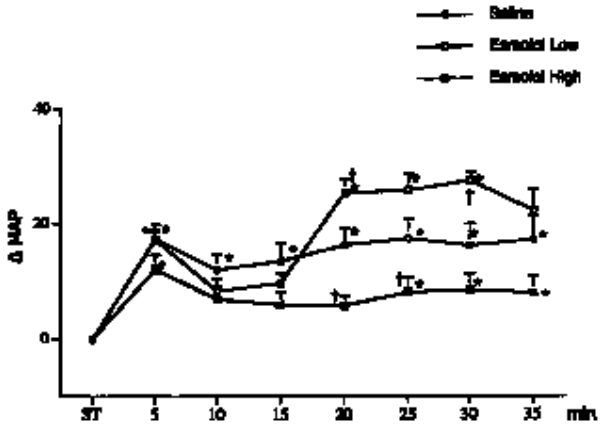

FIGURE 3 Mean arterial blood pressure changes following saline (n=9), esmolol low (n=7) and esmolol high (n=9) in rats subjected to formalin injection. Data are expressed as actual changes from ST (mean ± SEM). ST = steady state. *$P < 0.05$ *vs* ST. †$P < 0.05$ *vs* saline

during steady-state infusions of low and high doses of esmolol and saline prior to formalin injection. Although no changes in mean arterial pressure and heart rate occurred during the infusion of saline, esmolol low and high doses produced decreases in mean arterial blood pressure by -10.6 ± 2.2 mm Hg and –12.8 ± 1.8 mm Hg, respectively and in heart rate by -55.7 ± 10.4 bt·min^{-1} and -64 ± 9.9 bt·min^{-1}, respectively. Figures 3 and 4 represent the changes in mean arterial pressure and heart rate in rats treated with saline and high and low doses of esmolol after formalin injections.

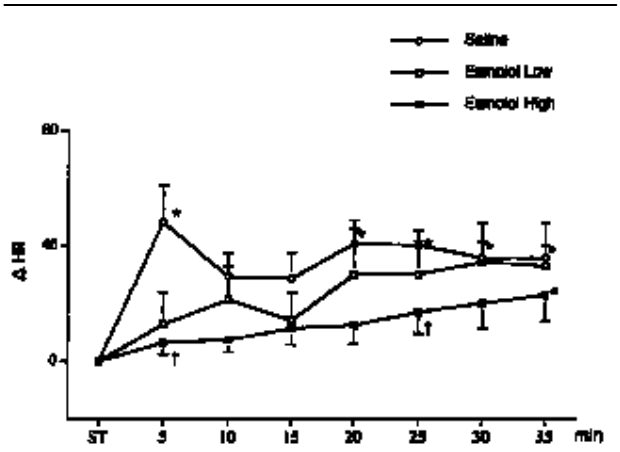

FIGURE 4 Heart rate changes following saline (n=9), esmolol low (n=7) and esmolol high (n=9) in rats subjected to formalin injection. Data are expressed as actual changes from ST (mean ± SEM). ST = steady state. *$P < 0.05$ *vs* ST. †$P < 0.05$ *vs* saline

Formalin injections induced an increase in mean arterial pressure in all groups. In the control group, there was an immediate and short lasting increase in mean arterial pressure (+17.4 ± 2.4 mm Hg) followed by a sustained, but less pronounced pressor effect. Esmolol did not affect the time course and magnitude of the initial increase in mean arterial pressure after formalin injection. However, the high esmolol treatment blunted the secondary rise in blood pressure. The increase in mean arterial pressure was significant only from 25 to 35 min. However, the magnitude was lower compared with control at 25 min. In contrast, the magnitude of the increase in mean arterial pressure in the low esmolol group was accentuated, especially at 20 and 30 min when compared with saline and from 20 to 35 min compared with high esmolol dose.

Formalin injection also induced a biphasic increase in heart rate in control conditions (Figure 4). In rats receiving saline, an immediate and transient increase in heart rate by 48.1 ± 12.7 bt·min^{-1}, followed by a secondary and longer lasting tachycardia - significant at 20 min to 35 min - was also recorded. Esmolol in low and high doses inhibited the formalin-mediated tachycardia during the first 30 min.

Discussion

Although previous studies have also shown that esmolol blunted the hypertension and tachycardia associated with several perioperative pain stimuli, including intubation,[17–20] emergence and extubation,[21]

the role played by esmolol in the modulation of pain during anesthesia has been questioned. Thus, in the absence of behavioural changes reflecting the intensity of the painful stimuli, it is always possible to consider that $ß_1$ blockers only mask inadequate anesthesia or reflect sedative properties of this group of drugs. To differentiate between sedation, inadequate anesthesia and analgesic properties of esmolol, our study has been conducted in conscious, free-moving rats. Although we did not perform specific motor and somotosensory testing, animals appeared to maintain normal behaviour before and during saline and esmolol administration. Therefore, our data suggest that esmolol is involved in the modulation of pain and some of the associated cardiovascular changes which have been shown to be independent of nociceptive behaviour and directly related to the level of the pain stimuli.[10]

The magnitude of the anti-nociceptive properties and associated cardiovascular properties of esmolol varied according to the parameter and the time course of the response following the formalin injection. Esmolol infused at low and high doses did not affect the behaviour changes recorded immediately after the formalin injection (Phase 1), whereas only the high esmolol dose was effective in decreasing lifting during the secondary response (Phase 2). Although esmolol also prevented the initial tachycardia (Phase 1), it has little effect on the increase in heart rate observed during the Phase 2. In contrast, the magnitude of the hypertension recorded immediately after formalin injection was not affected by esmolol, whereas the properties of esmolol on formalin-associated increase in mean arterial pressure during Phase 2 mimicked the effects on behaviour changes. It is unlikely that the effects of esmolol on pain-induced hypertension and tachycardia were related to the established ß blockade cardiovascular properties of esmolol. Thus, esmolol specifically diminished both the tachycardia during Phase 1 and the hypertension during Phase 2 that are associated with formalin injection.

Although intravenous esmolol produces its analgesic and associated cardiovascular properties peripherally, the blockade of central $ß_1$ adrenoreceptor may also be involved in the modulation of pain. Accordingly, propranolol is known to have centrally, as well as peripherally mediated properties.[22] In patients with coronary artery disease, Stanley *et al.* (1982) reported that chronic perioperative propranolol therapy decreased opioid requirements during anesthesia.[22] ONO 1101, a specific $ß_1$ blocker, injected intrathecally elicits a decrease in the nociceptive behaviour following formalin injection.[23] Thus, additional studies are required to better define the role of esmolol centrally administered in the modulation of pain.

Although human and animal studies have demonstrated an interrelationship between cardiovascular and pain regulatory systems,[24] our data also support the concept of independence between both pain and cardiovascular changes, especially with respect to the role played by esmolol. As a result, esmolol affected the pain behaviour following formalin injection. However, esmolol infused in low and high doses inhibited the formalin-mediated tachycardia whereas MAP increased at low esmolol dose only.

Conclusions

Our data indicate that esmolol has direct analgesic properties in rats following the injection of formalin.

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