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INSTRUMENTS AND TECHNIQUES

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Cardiovascular effects of fentanyl in conscious rats

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Abstract The polymicrobial sepsis induced by cecal ligation and puncture (CLP) in the rat is widely used in shock research. For ethical reasons, narcotic analgesics are often administered in this model, with the potential risk of confounding effects. In conscious non-septic rats, we investigated the cardiovascular effects of a continuous i.v. infusion of fentanyl (20 µg/kg per h) administered with fluid loading (10 ml/kg per h) for 24 h, a regimen commonly applied in rat CLP. Animals were randomly allocated to receive analgesia with fluid loading (Fentanyl group), or fluid loading alone (Control). All endpoints were assessed after 24 h of infusion. At that time, Control animals had mild respiratory alkalosis, which was essentially abolished by fentanyl. Analgesia mildly elevated the plasma norepinephrine levels [median (interquartile range): Control 232 pg/ml (0–292), Fentanyl 302 pg/ml (234–676), P=0.045] but was devoid of any effect on blood pressure, heart rate, cardiac output (mean ±SD: Control 388±61 ml/kg per min, Fentanyl 382±62 ml/kg per min, P=0.87) and indices of left ventricular function derived from high-fidelity recordings of left ventricular pressure (dP/dt_{max}: Control 11782±2324 mmHg/s, Fentanyl 12107±2816 mmHg/s, P=0.77). In ex vivo experiments carried out immediately after animal sacrifice, no differences were noted between the Control and Fentanyl groups in the sensitivity of endothelium-intact aortic rings to norepinephrine-induced vasoconstriction ($-\log EC_{50}$: Control 8.78±0.28, Fentanyl 8.83±0.26, P=0.52) or acetylcholine-induced vasodilatation ($-\log EC_{50}$: Control 7.00±0.37, Fentanyl 7.06±0.26±0.53, P=0.75). In conclusion, the present data provide no contraindication, and even some support for the ethical use of a high dose i.v. infusion of fentanyl in cardiovascular studies of conscious catheterized rats undergoing CLP or other painful procedures.

Keywords Analgesia · Hemodynamics · Left ventricular function · Opioids · Rats · Vascular endothelium · Vasoconstriction · Vasodilatation

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Introduction

The polymicrobial sepsis induced by cecal ligation and puncture (CLP) in the rat has gained wide popularity in shock research. Its main advantage compared to models based on the acute administration of endotoxin consists in more closely mimicking key events observed in septic patients, in particular the hyperdynamic circulation [13, 28, 48]. In CLP, sepsis develops subacutely, so that the required observation period is too prolonged to allow the full experiment to run under general anesthesia. This in itself is an advantage, since confounding effects related to the use of anesthetic agents [17] are avoided. However, in a large number of studies with the CLP model, continuous i.v. analgesia with opioid analogs in relatively large doses has been administered for ethical reasons [12, 13, 15, 25, 26, 27, 28, 30, 40, 41, 44, 47, 48]. The specific physiological effects of these regimens have not been documented, despite the fact that key endpoints examined in several of these studies could theoretically be influenced. In particular, it is well known that opioid agonists may induce both venous and arterial vasodilatation, and so alter the loading conditions of the heart [3, 43]. Furthermore, there are opioid receptors in the myocardium [4], the stimulation of which may either increase [31, 34] or decrease contractility [34, 46]. In addition, narcotic analgesics have the ability to stimulate

the release of nitric oxide by the vascular endothelium [8, 42], a consideration of interest in view of the importance of endothelial dysfunction in the pathogenesis of sepsis.

The present work was designed to characterize in the rat the cardiovascular effects of continuous intravenous analgesia, with special regard to in vivo left ventricular function and to vascular reactivity assessed ex vivo. The agent tested was fentanyl, which is most commonly used in rat CLP [12, 13, 15, 27, 28, 30, 40, 41, 44, 47, 48]. The rate of administration was the highest one reported in these studies. For ethical reasons, the investigation was restricted to nonseptic conditions.

Materials and methods

The study was in accordance with Swiss laws and approved by the local review board on animal experimentation. It was divided into two separate experiments, each conforming to the same design, with essential differences relating to endpoints only.

Experiment 1

Twenty-five male adult Wistar rats (IFFA CREDO, Lyon, France) weighing between 300 and 400 g were randomly allocated to an experimental group (n=13) receiving a continuous i.v. infusion of fentanyl or to a control group (n=12) receiving an equivalent volume of vehicle alone.

Surgery

The rats were anesthetized with pentobarbital 50 mg/kg i.p. Polyethylene (PE) catheters were implanted into the femoral artery (PE50, with PE10 tip pushed into the abdominal aorta), the right jugular vein (PE50 with silicone tip), and the left ventricle (LV) through the right carotid artery. The LV catheter was specially designed to allow the subsequent passage of a miniature transducer-tipped probe for the high-fidelity recording of pressure, as shown in Fig. 1A and described in detail below. At the time of insertion, the catheter did not contain the probe, but was connected to an external pressure transducer in order to guarantee tip placement inside the LV. All catheters were tunneled subcutaneously, exteriorized at the neck, and ran through a protective spring affixed to the skin by means of a Teflon anchoring flange on one end and to a swivel adapter on the other. For our application, it was critical to avoid a sharp bend of the left ventricular catheter at the exit site. For this reason, we used a special technique to affix the anchoring flange at some distance from the skin (Fig. 1B).

The crural and cervical incisions were closed in one layer, and the animals were left to awaken, in an individual cage.

Protocol

On termination of anesthesia, the jugular venous catheter was connected to a syringe pump (Perfusor-Secura, Braun, Switzerland) containing isotonic saline with or without 2 µg/ml fentanyl. The i.v. infusion was started and run continuously for 24 h at a rate of 10 ml/kg per h, making up for a fentanyl dose of 20 µg/kg per h, in accordance with the analgesic scheme followed in several studies of CLP [15, 40]. Starting the fentanyl administration immediately after surgery, rather than waiting for post-operative stabilization, was part of the effort to imitate conditions prevailing in CLP studies, where catheter insertion and cecal perforation are usually grouped in a single operation [12, 13, 15, 27, 28, 30, 40, 41, 44, 47, 48].

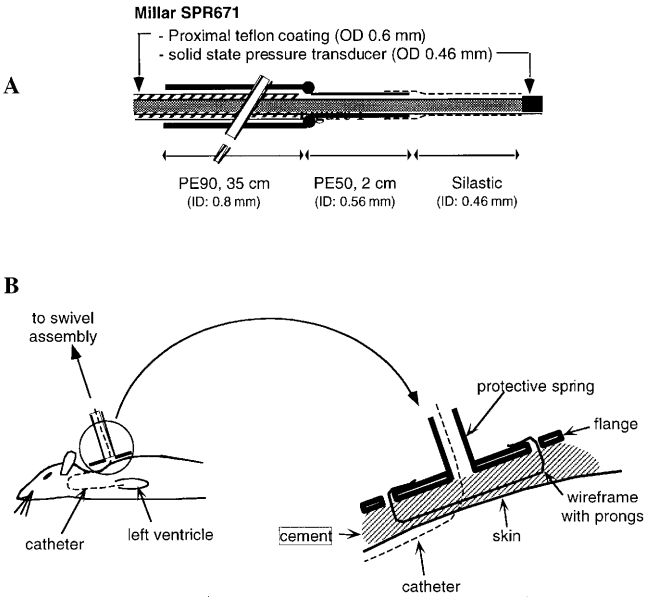

Fig. 1A, B Technical details concerning left ventricular catheterization in the conscious rat. **A** Construction of left ventricular catheter designed to allow the temporary introduction of a Millar transducer-tipped probe. The section of PE90 tubing is required to allow passage of the probe. Only the PE50 is intravascular. (*OD* Outer diameter.) **B** Attachment of the protective spring to the rat's neck. To minimize catheter curvature at this site, the Teflon flange used to affix the protective spring to the rat's neck is held in place at some distance from the skin, by means of a four-pronged wire frame embedded in cement

Food and tap water were provided ad libitum during the entire observation period.

Twenty-four hours after the onset of infusion, taking care to chose a moment when the animal was resting quietly and to avoid the making of any noise, respiratory rate was noted by counting the breaths during 30 s. The femoral catheter was connected to a pressure transducer for the measurement of blood pressure (BP) and heart rate (HR). High-fidelity recording of LV pressure was carried out next, as described below. Finally, arterial blood was drawn through the left ventricular catheter for the measurement of blood gases, lactate, and plasma nitrate. The latter is an index of whole-body production of nitric oxide. Nitrate was determined by a spectrophotometric assay based on the oxidation of NADPH during the enzymatic conversion of nitrate to nitrite by a nitrate reducatase, as previously described [24].

High-fidelity recording of left ventricular pressure in the conscious rat

Following calibration with a mercury manometer and zeroing in a water bath, a miniature transducer-tipped pressure probe (Mikro.Tip SPR 671, Millar, external diameter of the distal part 1.4F=0.46 mm) was introduced into the LV from the proximal end of the LV catheter, using an O-ring valve to avoid the reflux of blood. In so doing, the operator did not touch the rat, nor did his hands enter the cage. Thus, there was no need for a restraining tube. The animal did not even appear aware of probe passage. The tip transducer could always be advanced to <5 cm (usually <5 mm) of the LV catheter distal end, as determined from distance marks made on the Millar probe.

The LV pressure signal was digitized at 2 kHz and stored on computer disk for the subsequent calculation of the maximum systolic LV pressure (LVP_{max}), the LV end-diastolic pressure (LVEDP), the maximum positive rate of change in LV pressure during isovolumic contraction (dP/dt_{max}), the minimum negative

rate of change in left ventricular pressure during isovolumic relaxation (dP/dt_{min}), and the time constant of isovolumic left ventricular relaxation (tau). The latter was obtained from the least-square fit of an exponential function to the isovolumic descent of LV pressure. For all these calculations, we used the Heart Beat Program (San Diego, CA) [33].

Studies in isolated aorta

Animals were sacrificed with 100 mg/kg pentobarbital i.v. The thoracic aorta was excised and placed in phosphate-buffered saline at 4°C. Two rings of length between 2 and 3 mm were cut. The endothelium was removed with a thin wire in one of them, and left untouched in the other. The two rings were mounted in parallel in organ chambers for the measurement of isometric force (Multi Myograph System, model 610M, JP Trading, Denmark). The chambers were filled with Earle's Balanced Salt Solution (EBSS, Sigma) gassed at 37°C with 6% CO_2 + 94% O_2, pH 7.4. EBSS had the following composition (in mM): NaCl 116.3, KCl 5.3, $NaHCO_3$ 26.1, $CaCl_2$ 1.8, NaH_2PO_4 1.0, $MgSO_4$ 0.8, D-glucose 5.5, phenol red Na 0.03 (as pH indicator).

The rings were stretched and left to rest for 30 min. The applied preload was 90% of the circumference which, according to Laplace's law, would correspond to an equivalent transmural pressure of 100 mmHg. The maximal active tension (T_{max}) was determined by exposing the vessels to 80 mM KCl for a duration of 15 min, sufficient for stabilization. The solution used for that purpose was identical in composition to EBSS, except for the isosmolar substitution of KCl for NaCl. Following the washout of KCl (30 min), the rings were exposed to norepinephrine (NE), starting at a concentration of 10^{-9} M, with sequential 3.16-fold increases until the developed active tension was between 60% and 90% of T_{max}. The relaxation response to the endothelium-dependent vasodilator acetylcholine (ACh) was then recorded. To that effect, the rings were exposed sequentially to ACh concentrations of 10^{-8}, 10^{-7}, 10^{-6} and 10^{-5} M.

From the recorded data, the following quantities were computed: the NE concentration required for a contractile response equal to 50% of T_{max} (EC_{50}[NE]); the maximum relaxation induced by ACh, expressed as a percentage of the precontraction tension (R_{max}[ACh]), and the concentration of ACh required to halve the precontraction tension (EC_{50}[ACh]).

Experiment 2

This experiment was carried out to complement the previous one by the determination of further endpoints, especially cardiac output and the plasma levels of NE. In the following description, unnecessary repetitions of features that were kept identical between experiments will be avoided. A total of 19 rats were randomly allocated to the control (n=10) and the experimental groups (n=9).

Surgery

Anesthesia for surgery was with inhalation of 2% halothane in oxygen, which is more convenient than i.v. administration of pentobarbital. At the time of Experiment 1, our laboratory lacked a facility for the disposal of volatile anesthetics. In view of the 24-h interval from operation to actual measurements, we assumed that the use of different types of anesthesia in the two experiments would be of no consequence.

Only two vascular catheters were placed, one into the right jugular vein and one into the ascending aorta through the right carotid artery. The arterial catheter was a PE90 (outer diameter 1.26 mm) with a tip thinned to allow insertion. A thermistor wire dismounted from a used Swan-Ganz catheter (7F, Edwards Laboratories) ran through the lumen, all the way to the tip. The lumen was filled with saline containing 20 U/ml heparin. The catheter was advanced 3 cm from the vascular incision, a distance shown by experience to guarantee thermistor location within the ascending aorta.

Protocol

The endpoints, collected 24 h after the start of the fentanyl or saline-only infusion, were: respiratory rate, arterial BP, central temperature (°C), thermodilution cardiac output, plasma levels of catecholamines, arterial blood gases and lactate. Care was taken to obtain physiologic data and blood samples when the animal was resting quietly.

Thermodilution cardiac output in the conscious rat

The measurement of cardiac output conformed to the technique previously applied to the anesthetized rat in our laboratory [37]. Thermistor probe placement is described above. At the time of measurement, the thermistor wire was connected to a cardiac output computer (COM-1, Edwards), 0.15 ml of room temperature isotonic saline was injected into the right jugular vein, and the thermodilution curve was recorded on paper. Cardiac output in an individual animal was the mean of four to six determinations made at least 90 s apart. The values corresponding to irregular thermodilution waveforms were excluded. The coefficient of variation of these multiple determinations was always <20% (mean ±SD: 8.6±3.6%).

Plasma levels of catecholamines

Special precautions were taken at the time of blood sampling, in order to avoid potential artifacts related to the triggering of baroreflexes or the rapid degradation of catecholamines ex vivo. When the measurement of cardiac output was completed, the thermistor wire was quickly withdrawn, allowing for the easy efflux of arterial blood. Two 300-µl samples were collected in heparinized microtubes and centrifuged at 4000 rpm for 15 min at 4°C, to provide two 125-µl plasma aliquots which were stored at –80°C. The time interval from the sampling of blood to the freezing of plasma did not exceed 20 min. The concentration of NE was subsequently determined in the plasma aliquot obtained from the first sample, the second one being kept as a back up. Samples for the determination of arterial blood gases were collected after those for NE.

Catecholamines were determined by liquid chromatography with amperometric detection as previously described [16]. 0.125 ml of serum or standard, added with dihydroxybenzylamine (Sigma, Buchs, Switzerland) as internal standard, was extracted on activated alumina (Al_2O_3) at pH 8.6. The alumina was allowed to settle and the supernatant aspirated followed by three washes with water. The catecholamines were then eluted by 120 µl of a mixture of acetic acid (0.2 M) and phosphoric acid (0.04 M, 8/2, v/v) and 100 µl was injected into the chromatography system (Alliance, Waters 2690 System). The separation was achieved on a reversed phase column (Nucleosil 5 µm C-18, 25 cm × 4.6 mm, Macherey-Nagel, Oesingen, Switzerland) using a mobile phase containing a phosphate buffer at pH 3.5 with sodium octyl sulfonate, as an ion-pairing agent, and acetonitrile. The electrochemical detector (Decade, from Antec, Basel) was set at +0.8 V. Recovery of catecholamines from alumina was 75%. The limit of quantification was set at 6 pg NE and epinephrine per injection, corresponding to 80 pg/ml of serum.

Statistical analysis

In each experiment, endpoints were compared between groups by means of the Student's t-test for unpaired samples, with Welch adjustment for unequal variances when appropriate. Since the distribution of plasma NE data was highly skewed, a Mann–Whitney test was used in this instance. The alpha level of all tests was set at 0.05.

All actual P values are reported. Data are summarized as mean ±SD.

Table 1 Pulmonary gas exchange and blood lactate

	Control	Fentanyl	*P* value
Experiment 1			
Respiratory rate (c/min)	101±13	75±6	<0.00001
P_aO_2 (mmHg)	95±8	82±10	0.0016
P_aCO_2 (mmHg)	33±4	39±3	0.0006
Arterial pH	7.53±0.06	7.45±0.03	0.0003
Blood lactate (mM)	0.92±0.41	0.62±0.12	0.02
Experiment 2			
Respiratory rate (c/min)	88±13	73±11	0.015
P_aO_2 (mmHg)	94.7±5.0	81.4±9.2	0.002
P_aCO_2 (mmHg)	34.8±5.0	41.0±4.7	0.012
Arterial pH	7.49±0.03	7.44±0.03	0.002
Blood lactate (mM)	0.66±0.30	0.79±0.33	0.38

Table 2 Hemodynamic data. (*BP* Arterial blood pressure, LVP_{max} maximum left ventricular pressure in systole, *LVEDP* left ventricular end-diastolic pressure, dP/dt_{max} maximum positive rate of change in left ventricular pressure during isovolumic contraction, dP/dt_{min} minimum negative rate of change in left ventricular pressure during isovolumic relaxation, *tau* time constant of isovolumic left ventricular relaxation)

	Control	Fentanyl	*P* value
Experiment 1			
Heart rate (beats/min)	421±60	416±44	0.86
Mean BP (mmHg)	115±13	112±14	0.63
LVP_{max} (mmHg)	136±14	135±16	0.86
LVEDP (mmHg)	13±7	12±5	0.64
dP/dt_{max} (mmHg/s)	11782±2324	12107±2816	0.77
dP/dt_{min} (mmHg/s)	–10395±1306	–9532±2906	0.38
tau (ms)	8.2±3.2	10.5±3.0	0.11
Experiment 2			
Heart rate (beats/min)	354±25	361±29	0.55
Mean BP (mmHg)	127±13	118±8	0.10
Cardiac index (ml/min per kg)	388±61	382±62	0.87
Stroke volume (ml)	1.11±0.17	1.07±0.22	0.71
Central temperature (°C)	36.6±1.7	36.3±1.4	0.61

Results

As shown in Table 1, the control rats had a moderate respiratory alkalosis, which appeared less marked in Experiment 2, compared with Experiment 1. In contrast, animals treated with fentanyl had a practically normal acid–base status of the arterial blood, along with a significantly lower respiratory rate. These effects of analgesia were consistently observed in both experiments. Blood lactate was within the normal range in all conditions, although significantly lower with fentanyl treatment in Experiment 1. The P_aO_2 was significantly lower in the treated, compared with control groups, but all animals remained in the normoxemic range.

Hemodynamic data are shown in Table 2. Animals in Experiment 1 were somewhat tachycardic, while those in Experiment 2 had heart rates in complete agreement with usually reported values [9, 19]. The mean BP was within the normal range in all conditions. In Experiment 1, the indices of LV function derived from the high-fidelity recording of LV pressure were very consistent with data published by others who used similar instrumentation in conscious, unrestrained resting rats [9, 19]. The same statement applies to the cardiac output measured in Experiment 2 [12, 30]. Analgesia had no detectable hemodynamic effect in any of these experiments. As provided by the thermistor catheter in Experiment 2, central temperature was in the lower normal range for conscious rats [7] (Table 2, last line), and was not affected by analgesia. The differences in heart rate and respiratory rate between Experiment 1 and Experiment 2 are probably related to the fact that animals were not comparably instrumented. A less likely explanation in view of the precautions taken (see Materials and methods) would be

Table 3 Vascular reactivity (Experiment 1). Rings of abdominal aorta mounted in the myograph in Experiment 1. Two rings per rat, with the endothelium intact and removed, respectively. T_{max} Maximum tension developed in response to 80 mM KCl, *–log EC_{50} [NE]* negative logarithm of the concentration of norepinephrine which induced a contractile response equal to 50% of T_{max}, R_{max} *[ACh]* maximum relaxation induced by acetylcholine in rings precontracted with NE (expressed as a percent of precontraction tension), *–log EC_{50} [ACh]* negative logarithm of the concentration of ACh required to reduce the precontraction tension by 50%

	Control	Fentanyl	*P* value
Rings with endothelium			
T_{max} (mN/mm)	6.82±0.84	6.81±1.32	0.43
–log EC_{50} [NE]	8.01±0.22**	8.03±0.50**	0.52
R_{max} [ACh] (%)	96.9±3.9**	93.8±7.2**	0.43
–log EC_{50} [ACh]	7.00±0.37	7.06±0.53	0.75
Rings without endothelium			
T_{max} (mN/mm	6.08±1.23	6.05±1.02	0.43
–log EC_{50} [NE]	8.78±0.28	8.83±0.26	0.52
R_{max} [ACh] (%)	7±7	6±6	0.43
–log EC_{50} [ACh]	–	–	–

P values in the *last column* refer to comparisons between the Control and Fentanyl groups. **P<0.001 compared with rings without endothelium

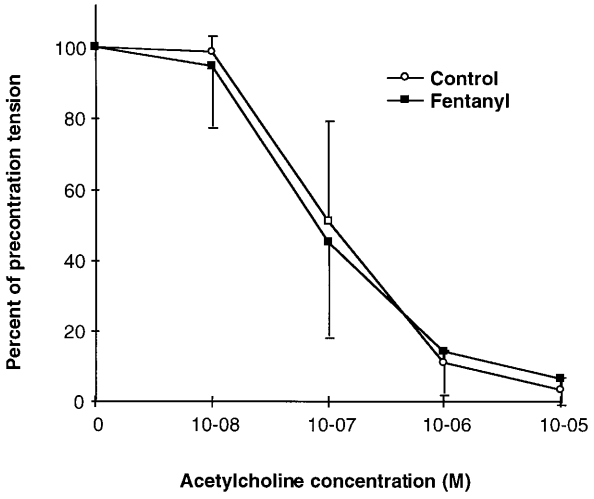

Fig. 2 In vitro relaxation of endothelium-intact aortas in response to acetylcholine (Experiment 1). Error bars indicate SD

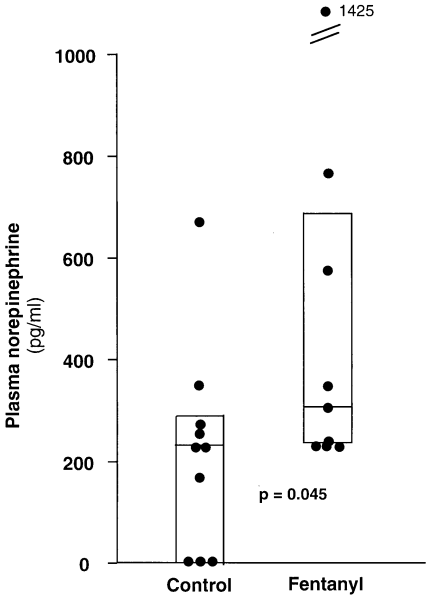

Fig. 3 Plasma concentration of norepinephrine (Experiment 2). Three animals in the Control group had values below the detection limit of 80 pg/ml (plotted as zeros)

the potential influence of the environment, in particular ambient noise, on these variables [39], since the two experiments were not performed by the same investigator. If at all operative, this factor would have no bearing on the comparisons of interest in our study, namely between conditions with and without fentanyl, because each of the two experiments was carried out entirely by a single investigator (F.B. in Experiment 1, and D.G. in Experiment 2).

Considering the potential influence of environmental factors, in particular ambient noise, on these variables [39], one might also note that the two experiments were not carried out by the same operator.

The contractile behavior of isolated aortas (Experiment 1) was entirely consistent with published observations [14, 20, 29, 45, 47] and was not influenced by treatment with fentanyl (Table 3). In particular, analgesia had no effect on the endothelium-dependent relaxation induced by ACh (Table 3, Fig. 2).

The plasma levels of nitrate obtained in Experiment 1 were 16.8±12.6 µM in the Control and 10.8±4.7 µM in the Fentanyl group (P=0.20).

As determined in Experiment 2, the median plasma level of NE was 232 pg/ml (lower quartile below the detection limit of 80 µg/ml, upper quartile 292 pg/ml) in the Control group, in agreement with values previously found in this laboratory in a study of conscious unrestrained rats [49]. As shown in Fig. 3, plasma NE was mildly augmented by treatment with fentanyl (median 302 pg/ml, lower quartile 234 pg/ml, upper quartile 676 pg/ml, P=0.045). Plasma epinephrine was below the detection limit of 80 pg/ml in all animals.

Discussion

This investigation sought to identify potential confounding cardiovascular effects of fentanyl, an analgesic that is widely applied in studies of sepsis employing CLP in the rat [12, 13, 15, 27, 28, 30, 40, 41, 44, 47, 48]. Accordingly, the dosage rate and route of administration of fentanyl, the duration of observation for its effect, and the fluid load (10 ml/kg/h) administered were dictated by these previous studies. Based on previous observations in humans [35, 43], we expected that the cardiovascular effects of analgesia under these conditions would be minimal. Indeed, no impact was found on systemic hemodynamics, left ventricular function, or vascular reactivity of aortas examined ex vivo.

Mild hyperventilation was present in the control animals, as shown by the moderate hypocapnia and slightly higher P_aO_2 observed in this group in both experiments (Table 1). Hyperventilation could have been induced by the physical discomfort and/or the stress related to various aspects of the experimental setup, in particular the implantation of vascular catheters. Furthermore, respiratory stimulation could have come from some degree of pulmonary vascular congestion, induced by the fluid load received, combined in Experiment 1 with alteration in left ventricular function by the LV catheter. Nevertheless, in the absence of hypoxemia (Table 1) and clear elevation of LVEDP (Table 2), pulmonary congestion due to left heart failure is unlikely.

Whatever the cause of hyperventilation, it was markedly blunted by treatment with fentanyl, indicating that the dosing regimen used here did have central effects. The lack of complete tolerance to the respiratory depressant action of a mu-receptor agonist after 24 h of continuous i.v. administration is consistent with previous observations in the rat [1, 5].

Opiates, notably fentanyl, are known for an ability to induce bradycardia through vagal stimulation [3]. The lack of bradycardia in the fentanyl-treated rats (Table 2) might be related to the continuous infusion, as opposed to bolus administration, because the negative chronotropic action of opiates is inversely related to speed of administration [3].

To rule out any effect of fentanyl on cardiac contractility in the chosen experimental conditions, we carried out an assessment of the dP/dt_{max}. The required intraventricular placement of a high-fidelity pressure sensor has rarely been achieved in the conscious rat [9, 38]. These authors inserted the Millar probe under anesthesia and left it in place while consciousness and motility were regained. In this method, the necessity to tape the bulky connector to the back of the rat adds to the restraint imposed on the animal. A further potential problem is the risk of damage inflicted on the expensive device, especially if left in situ for a prolonged period. Probe introduction through the proximal part of a LV catheter obviates these disadvantages. The latter principle was originally described in conscious rats by Flaim and coworkers [10, 11], but the transducers available at that

time were too bulky to be placed inside PE50 tubing and thus could not be brought closer than a few centimeters to the LV. We removed this limitation by using the newly developed miniature Millar probe. The values of dP/dt_{max} thus measured in Experiment 1 agreed with those obtained in conscious rats with operatively inserted high-fidelity transducers [9, 38]. Treatment with fentanyl had no effect on myocardial contractility as reflected by these values (Table 2). Isovolumic (pre-ejection) dP/dt_{max} is independent of afterload but depends on ventricular preload, or end-diastolic volume. However, the identity of LVEDP in Experiment 1 supports the equivalency of preload in the control and treated rats. We acknowledge that a constant LVEDP does not in itself guarantee an identical LV preload, or myocardial fiber stretch, due to potential variations in the diastolic properties (relaxation kinetics, active and passive stiffness) of the myocardium. The lack of significant difference in dP/dt_{min} and tau (control versus fentanyl, Table 2) would not favor an effect of this analgesic on LV relaxation kinetics. Taken together, we believe our data strongly support the lack of effect of fentanyl on LV contractility. This interpretation is consistent with recent observations in isolated canine hearts [22], showing that concentrations of fentanyl up to 240 ng/ml fail to modify LV end-systolic elastance in this preparation. Furthermore, while fentanyl is essentially a mu agonist [36], kappa but not mu opioid receptors have been identified in the rat myocardium [23, 34].

The fact that systemic hemodynamics were not detectably affected by the continuous infusion of fentanyl at 24 h (Table 2) could reflect the development of tolerance [6]. Alternatively, a compensatory activation of the sympatho-adrenergic system might have come into play, a possibility suggested by the higher plasma level of NE in the treated compared with the control group (Fig. 3). In addition, the sizable amount of i.v. fluid received in the course of the experiment might have been protective. Thus, one should caution against the extrapolation of the present results to conditions of less intensive fluid loading.

Opioids may induce vasodilatation by a variety of mechanisms, including histamine release [36], stimulation of nitric oxide production by the endothelium [42], and interference with alpha-adrenergic receptor signal transduction [21]. In vitro, fentanyl has no effect on cultured endothelial cells [42], but is able to relax endothelium-denuded rat aortas precontracted with an alpha-adrenergic agonist [21] or to increase the basal tone of dog coronary arteries [18]. To our knowledge, the effects of prolonged opioid administration in vivo on vascular reactivity examined in vitro have not been documented. In the present study, the reactivity of isolated aortas was not affected by treatment with fentanyl (Table 3, Fig. 2). Whether explained by washout of the opioid after excision or by other factors, this observation indicates that the present regimen of analgesia may be given to rats without undue concern for subsequent interference with aortic reactivity, when examined ex vivo. Further experiments are required before extending the present finding to other vessels. In this context, it is noteworthy that plasma nitrate did not differ between groups, suggesting that fentanyl analgesia did not stimulate the expression of inducible nitric oxide synthase. This would be in contrast to pentobarbital anesthesia, which has been shown in the rat to depress vascular contractility by this very mechanism [17].

In conclusion, the present data provide no contraindication to the ethical use of a high dose i.v. infusion of fentanyl in conscious catheterized rats undergoing CLP. This statement is valid for experiments conducted under substantial fluid loading and lasting 24 h as is the case in most sepsis studies with this model. The reasons are as follows. First, there is no gross encroachment of this regimen of analgesia per se on the cardiovascular physiology of the animal. One might argue that this apparent innocuousness is obtained at the cost of utilizing part of the adaptation reserve, as possibly reflected by the observed increase in plasma NE (Fig. 3). However, any reduction in cardiovascular reserve due to fentanyl would probably be of minor significance in the 24 h CLP model, because cardiovascular collapse does not occur in this setting, as reported by many studies, including one by our group [2, 28, 48]. Finally, if only because of its potential effects on organ blood flow [32], the respiratory alkalosis found in conscious catheterized rats not receiving fentanyl could in itself influence the time course of sepsis, such that its blunting might in fact be a methodologically useful side-effect of analgesia.

As a final note, two limitations of the present study must be underscored. First, we have not assessed the time course of potential cardiovascular effects related to analgesia. It remains possible that observations made especially earlier (see previous remark on tolerance) might have differed from those presented. Our emphasis on the 24 h time point is justified by its common use in studies of sepsis with the rat CLP model. Second, fentanyl was only evaluated in nonseptic animals. In view of the emerging role of opioid receptors in the modulation of the immune response, our results do not imply equivalent behaviors of the CLP model in presence or absence of analgesia. Inclusion of analgesia in the experimental conditions is clinically relevant in view of the frequent use of opioids in critically ill septic patients.

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References

1. Ayesta FJ, Florez J (1989) Tolerance to respiratory actions of sufentanil: functional tolerance and route-dependent differential tolerance. J Pharmacol Exp Ther 250:371–378
2. Baechtold F, Scott JA, Markert M, Mehta S, McCormack DG, Anglada A, Galaud D, Vaglio, Waeber B, Feihl F (2001) Does the activation of poly (ADP-ribose) synthetase mediate tissue injury in the sepsis induced by cecal ligation and puncture? Shock 16:137–142

3. Bailey PL, Stanley TH (1994) Intravenous opioid anesthetics. In: Miller RD (ed) Anesthesia, 4th edn. Churchill Livingstone, New York, pp 291–387
4. Barron BA (1999) Opioid peptides and the heart [editorial comment]. Cardiovasc Res 43:13–16
5. Burton CK, Ho IK, Hoskins B (1990) Evidence for involvement of cyclic GMP phosphodiesterase in morphine tolerance. J Pharmacol Exp Ther 252:104–111
6. Doorley BM, Knight VV, Spaulding TC (1988) Development of acute tolerance to the cardiorespiratory effects of alfentanil after subsequent bolus injection in conscious rats. Life Sci 43: 365–372
7. Farris EJ, Griffith JQ (1967) The rat in laboratory investigation. Hafner, New York
8. Fimiani C, Mattocks D, Cavani F, Salzet M, Deutsch DG, Pryor S, Bilfinger TV, Stefano GB (1999) Morphine and anandamide stimulate intracellular calcium transients in human arterial endothelial cells: coupling to nitric oxide release. Cell Signal 11:189–193
9. Fitzsimons DP, Bodell PW, Herrick RE, Baldwin KM (1990) Left ventricular functional capacity in the endurance-trained rodent. J Appl Physiol 69:305–312
10. Flaim SF, Nellis SH, Toggart EJ, Drexler H, Kanda K, Newman ED (1984) Multiple simultaneous determinations of hemodynamics and flow distribution in conscious rat. J Pharmacol Methods 11:1–39
11. Flaim SF, Stranieri MT, Gill A, Carson JR, Brannan MD (1988) Effects of the novel calcium channel blocker, McN-5691, on cardiocirculatory dynamics and cardiac output distribution in conscious spontaneously hypertensive rat. J Cardiovasc Pharmacol 11:489–500
12. Fox GA, Paterson NAM, McCormack DG (1994) Effect of inhibition of NO synthase on vascular reactivity in a rat model of hyperdynamic sepsis. Am J Physiol 267:H1377–H1382
13. Fox GA, Paterson NA, McCormack DG (1996) Cyclooxygenase inhibition and vascular reactivity in a rat model of hyperdynamic sepsis. J Cardiovasc Pharmacol 28:30–35
14. Furchgott RF, Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373–376
15. Gocan NC, Scott JA, Tyml K (2000) Nitric oxide produced via neuronal NOS may impair vasodilatation in septic rat skeletal muscle. Am J Physiol 278:H1480–H1489
16. Grouzman E, Borgeat A, Fathi M, Gaillard RC, Ravussin P (2000) Plasma and cerebrospinal fluid concentration of neuropeptide Y, serotonin, and catecholamines in patients under propofol or isoflurane anesthesia. Can. J. Physiol. Pharmacol. 78:100–107
17. Hoque AM, Marczin N, Catravas JD, Fuchs LC (1996) Anesthesia with sodium pentobarbital enhances lipopolysaccharide-induced cardiovascular dysfunction in rats. Shock 6:365–370
18. Introna RP, Bridges MT Jr., Yodlowski EH, Grover TE, Pruett JK (1995) Direct effects of fentanyl on canine coronary artery rings. Life Sci 56:1265–1273
19. Irlbeck M, Muhling O, Iwai T, Zimmer HG (1996) Different response of the rat left and right heart to norepinephrine. Cardiovasc Res 31:157–162
20. Julou-Schaeffer G, Gray GA, Fleming I, Schott C, Parratt JR, Stoclet JC (1990) Loss of vascular responsiveness induced by endotoxin involves L-arginine pathway. Am J Physiol 259: H1038–H1043
21. Karasawa F, Iwanov V, Moulds RF (1993) Effects of fentanyl on the rat aorta are mediated by alpha-adrenoceptors rather than by the endothelium. Br J Anaesthiol 71:877–880
22. Kohno K, Takaki M, Ishioka K, Nakayama Y, Suzuki S, Araki J, Namba T, Suga H (1997) Effects of intracoronary fentanyl on left ventricular mechanoenergetics in the excised cross-circulated canine heart (revised publication). Anesthesiology 87:658–666
23. Krumins SA, Faden AI, Feuerstein G (1985) Opiate binding in rat hearts: modulation of binding after hemorrhagic shock. Biochem Biophys Res Commun 127:120–128
24. Liaudet L, Fishman D, Markert M, Perret C, Feihl F (1997) L-Canavanine improves organ function and tissue adenosine triphosphate levels in rodent endotoxemia. Am J Respir Crit Care Med 155:1643–1648
25. Lundblad R, Giercksky KE (1995) Effect of volume support, antibiotic therapy, and monoclonal antiendotoxin antibodies on mortality rate and blood concentrations of endothelin and other mediators in fulminant intra-abdominal sepsis in rats. Crit Care Med 23:1382–1390
26. Lundblad R, Sandven P, Giercksky KE (1995) The physical nature of a large bowel perforation predicts severity of the subsequent inflammatory response. Shock 3:455–461
27. Martin CM, Yaghi A, Sibbald WJ, McCormack D, Paterson AM (1993) Differential impairment of vascular reactivity of small pulmonary and systemic arteries in hyperdynamic sepsis. Am Rev Respir Dis 148:164–172
28. Martin M, Sibbald WJ (1994) Modulation of hemodynamics and organ blood flow by nitric oxide synthase inhibition is not altered in normotensive, septic rats. Am J Respir Crit Care Med 150:1539–1544
29. McKenna TM (1990) Prolonged exposure of rat aorta to low levels of endotoxin in vitro results in impaired contractility. J Clin Invest 86:160–168
30. Morisaki H, Sibbald W, Martin C, Doig G, Inman K (1996) Hyperdynamic sepsis depresses circulatory compensation to normovolemic anemia in conscious rats. J Appl Physiol 80: 656–664
31. Novakova M, Ela C, Barg J, Vogel Z, Hasin Y, Eilam Y (1995) Inotropic action of sigma receptor ligands in isolated cardiac myocytes from adult rats. Eur J Pharmacol 286:19–30
32. Nunn JF (1993) The effects of changes in carbon dioxide tension. In: Applied respiratory physiology, 4th edn. Butterworth, London
33. Palakodeti V, Oh S, Oh BH, Mao L, Hongo M, Peterson KL, Ross J Jr. (1997) Force-frequency effect is a powerful determinant of myocardial contractility in the mouse. Am J Physiol 273:H1283–H1290
34. Pei JM, Yu XC, Bian JS, Wong TM (1999) Acidosis antagonizes intracellular calcium response to kappa-opioid receptor stimulation in the rat heart. Am J Physiol 277: C492–C500
35. Peng PW, Sandler AN (1999) A review of the use of fentanyl analgesia in the management of acute pain in adults [Review]. Anesthesiology 90:576–599
36. Reisine T, Pasternak K (1996) Opioid analgesics and antagonists. In: Hardman JG, Limbird LE (eds) Goodman and Gilman's the pharmacological basis of therapeutics. McGraw-Hill, New York, pp 521–555
37. Rosselet A, Feihl F, Markert M, Gnaegi A, Perret C, Liaudet L (1998) Selective iNOS inhibition is superior to norepinephrine in the treatment of rat endotoxic shock. Am J Respir Crit Care Med 157:162–170
38. Salyers AK, Rozek LF, Bittner SE, Walsh GM (1988) Simultaneous determination of ventricular function and systemic hemodynamics in the conscious rat. J Pharmacol Methods 19: 267–274
39. Schlatter J, Zbinden G (1982) Heart rate- and ECG-recording in the rat by biotelemetry. Arch Toxicol [Suppl.] 5:179–183
40. Scott JA, McCormack DG (1999) Selective in vivo inhibition of inducible nitric oxide synthase in a rat model of sepsis. J Appl Physiol 86:1739–1744
41. Sielenkamper AW, Chin-Yee IH, Martin CM, Sibbald WJ (1997) Diaspirin crosslinked hemoglobin improves systemic oxygen uptake in oxygen supply-dependent septic rats. Am J Respir Crit Care Med 156:1066–1072
42. Stefano GB, Hartman A, Bilfinger TV, Magazine HI, Liu Y, Casares F, Goligorsky MS (1995) Presence of the mu3 opiate receptor in endothelial cells. Coupling to nitric oxide production and vasodilation. J Biol Chem 270: 30290–30293
43. Teeple E Jr. (1990) Pharmacology and physiology of narcotics. [Review]. Crit Care Clin 6:255–282

44. Tyml K, Yu J, McCormack DG (1998) Capillary and arteriolar responses to local vasodilators are impaired in a rat model of sepsis. J Appl Physiol 84:837
45. Umans JG, Wylam ME, Samsel RW, Edwards J, Schumacker PT (1993) Effects of endotoxin in vivo on endothelial and smooth-muscle function in rabbit and rat aorta. Am Rev Respir Dis 148:1638–1645
46. Xiao RP, Pepe S, Spurgeon HA, Capogrossi MC, Lakatta EG (1997) Opioid peptide receptor stimulation reverses beta-adrenergic effects in rat heart cells. Am J Physiol 272: H797–H805
47. Yaghi A, Paterson NAM, McCormack DG (1995) Vascular reactivity in sepsis: importance of controls and role of nitric oxide. Am J Respir Crit Care Med 151:706–712
48. Yu P, Boughner DR, Sibbald WJ, Keys J, Dunmore J, Martin CM (1997) Myocardial collagen changes and edema in rats with hyperdynamic sepsis. Crit Care Med 25:657–662
49. Zanchi A, Schaad NC, Osterheld MC, Grouzmann E, Nussberger J, Brunner HR, Waeber B (1995) Effects of chronic NO synthase inhibition in rats on renin-angiotensin system and sympathetic nervous system. Am J Physiol 268: H2267–H2273