Insulin effects on myocardial function and bioenergetics in L-bupivacaine toxicity in the isolated rat heart

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Summary

Background and objectives: A positive effect of insulin–glucose–potassium infusion in severe bupivacaine-induced cardiovascular collapse has been described *in vivo*. It has been speculated that an antagonistic influence of insulin on sodium channel inhibition, transient outward potassium current, calcium-dependent adenosine triphosphatase or even improved myocardial energetics may be responsible for this effect. Using an isolated heart model, we therefore sought to further elucidate insulin effects in L-bupivacaine-induced myocardial depression. *Methods:* An isolated rat heart constant-pressure perfused, non-recirculating Langendorff preparation was used. Hearts were exposed to L-bupivacaine 5 $\mu g\,mL^{-1}$ and insulin 10 $mIU\,mL^{-1}$. Heart rate, systolic pressure, the first derivative of left ventricular pressure ($+dP/dt$), coronary flow, double product, PR and QRS intervals were recorded. Hearts were freeze-clamped and high-performance liquid chromatography measurement of the total adenine nucleotide pool was performed. *Results:* L-Bupivacaine led to a significant decrease in heart rate, $+dP/dt$, systolic pressure, coronary flow and double product, and to an increase in PR and QRS. Insulin exerted a positive inotropic effect, significantly augmenting $+dP/dt$ and systolic pressure in both L-bupivacaine-treated and control hearts. Heart rate, coronary flow, total adenine nucleotides, PR and QRS were not significantly changed by the insulin intervention. *Conclusion:* Insulin did not have a significant effect on total adenine nucleotides in controls and in L-bupivacaine-treated hearts. However, it does exert a positive inotropic action in bupivacaine-induced myocardial depression. We conclude that the positive effect of insulin application lies in positive inotropic action and not in changes in total adenine nucleotides.

Keywords: ANAESTHETICS LOCAL, bupivacaine, toxicity; DRUG TOXICITY; HEART; INSULIN; ADENOSINE TRIPHOSPHATE.

Introduction

Extensive research has focused on the cardiotoxic effects of local anaesthetics, especially since Albright's review in 1979 describing a series of clinical cases of cardiovascular complications after application of bupivacaine [1]. Several novel interventions have been proposed for treatment of myocardial local anaesthetic toxicity (for review see [2]). Recently, two papers have evaluated the *in vivo* effects of insulin–glucose–potassium infusion in severe bupivacaine-induced cardiovascular collapse [3,4]. In the discussion of their work, the authors speculate that an antagonistic effect of insulin on sodium channel inhibition, transient outward potassium current or calcium-dependent adenosine

The results of this study were presented at the Wissenschaftliche Arbeitstage der Deutschen Gesellschaft für Anästhesie und Intensivmedizin in Würzburg (February 2005) and are part of the doctoral thesis of Silvi Hannack.

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triphosphatase may be responsible for this effect. Others believe that improved myocardial energetics play a key role in the insulin effect [5].

In this study we therefore sought to further describe insulin effects on the two main aspects of myocardial toxicity of local anaesthetics, namely negative inotropy and myocardial conduction block [6], in bupivacaine-induced myocardial depression and to measure myocardial adenine nucleotides in an isolated rat heart Langendorff model. Our hypothesis was that insulin has a positive inotropic effect in bupivacaine-induced myocardial depression and improves myocardial bioenergetics.

Methods

Preparation of isolated hearts

An isolated perfused, non-recirculating Langendorff rat heart preparation was used in our study. The investigation conformed to the Guide for the Care and Use of Laboratory Animals issued by the US National Institutes of Health and was approved by the local government authority (AZ 24-9168.24-1-2003-9). All experiments were conducted with Wistar rats (16–22 weeks old; 200–250 g, $n = 35$) purchased from Charles River (Sulzfeld, Germany). The animals were heparinized intraperitoneally ($1000\,U\,kg^{-1}$) to prevent the formation of intracoronary microthrombi [7] and were euthanized with $150\,mg\,kg^{-1}$ of intraperitoneal thiopental. Hearts were rapidly excised and perfusion was performed in a retrograde manner via the aorta at a constant perfusion pressure of 90 mmHg with a modified Krebs–Henseleit solution (KHS in $mmol\,L^{-1}$): NaCl 116, KCl 4.56, $MgSO_4$ 2.24, KH_2PO_4 1.18, $NaHCO_3$ 25.0, glucose 8.27, pyruvate 2.0, $CaCl_2$ 2.52. The solution was continuously bubbled with 95% oxygen and 5% carbon dioxide and pH was maintained at 7.35 ± 0.02. Arterial and venous PO_2 and PCO_2 (sampled via a catheter placed in the pulmonary artery) were measured at 0 and 15 min (AVL 990; Medical Instruments, Bad Homburg, Germany). Myocardial oxygen consumption (MvO_2, $\mu L\,min^{-1}\,g^{-1}$) was calculated from the arterial–venous difference of PO_2 ($\Delta PO_2 = P_{art}O_2 - P_{ven}O_2$) according to Fick's principle with the use of Bunsen's absorption coefficient ($\alpha = 0.036\,\mu L\,mmHg^{-1}\,mL^{-1}$) at 37°C as follows: MvO_2 ($\mu L\,min^{-1}\,g^{-1}$) $= \Delta PO_2 \times \alpha \times F$, where F denotes coronary flow ($mL\,min^{-1}\,g^{-1}$). All elements of the perfusion apparatus were water-jacketed and maintained at 37°C. In the spontaneously beating heart preparation, stable conditions were achieved in preliminary control hearts with minimal changes of inotropic parameters, left ventricular pressure and $+dP/dt$, in accordance with previous publications on the isolated heart model [8]. Left ventricular systolic pressure (LVP) and its first derivative $+dP/dt$ were continuously measured with a balloon catheter inserted into the left ventricle (Gould Inc. Instruments, Statham, USA) via the cut mitral valve. Diastolic left ventricular pressure (DVP) was adjusted to 5 mmHg. Coronary flow and coronary perfusion pressure were continuously measured by an in-line flow probe (Transonic Flowprobe; Transonic Systems Inc., New York, NY, USA) and a pressure transducer (Gould Nicolet, Erlensee, Germany) connected to the perfusion cannula 2 cm above the orifice of the coronary vessels. Haemodynamic parameters and derivatives (heart rate (HR), LVP, $+dP/dt$, coronary flow) and electrocardiogram (ECG) data (PR, QRS intervals) were continuously sampled and documented by a software system (PoNeMah, P3 plus Version 4, Gould LDS Test and Measurement LLC, OH, USA). All ECG data were crosschecked manually off-line to confirm correct assessment. Left ventricular developed pressure (LVDP) was calculated as LVP − DVP. The double product (DP) was calculated as follows: HR × LVDP. All infused compounds were applied through a stainless steel cannula placed in the aortic inflow line approximately 2 cm above the coronary orifices at a rate of $200\,\mu L\,min^{-1}$ or less (Precidor, Infors AG, Basel, Switzerland). The experimental protocol was started when LVP, $+dP/dt$ and HR had reached stable baseline values, i.e. 20 min after artificial perfusion has commenced.

Experimental protocol

After 20 min of the steady state perfusion, hearts were randomized to one of seven groups (five hearts per group, Figure 1).

Group 1: Perfusion with KHS for 10 min only.
Group 2: Perfusion with KHS for 15 min only.
Group 3: (Control) Perfusion with KHS for 20 min only.
Group 4: Switch to L-bupivacaine $5\,\mu g\,mL^{-1}$ after 10 min for 5 min.
Group 5: (L-Bupi) Switch to L-bupivacaine $5\,\mu g\,mL^{-1}$ after 10 min for 10 min.
Group 6: (Control Insulin) perfusion with KHS and insulin infusion ($10\,mIU\,mL^{-1}$) after 15 min for 5 min.
Group 7: (L-Bupi Insulin) At 10 min switch to L-bupivacaine $5\,\mu g\,mL^{-1}$ for 10 min and at 15 min additional infusion of insulin $10\,mIU\,mL^{-1}$ for 5 min.

In the L-Bupi Insulin group, insulin and L-bupivacaine were therefore applied simultaneously. Insulin was added via a precision infusion pump and the infusion speed was adapted depending on respective coronary flow to achieve a concentration

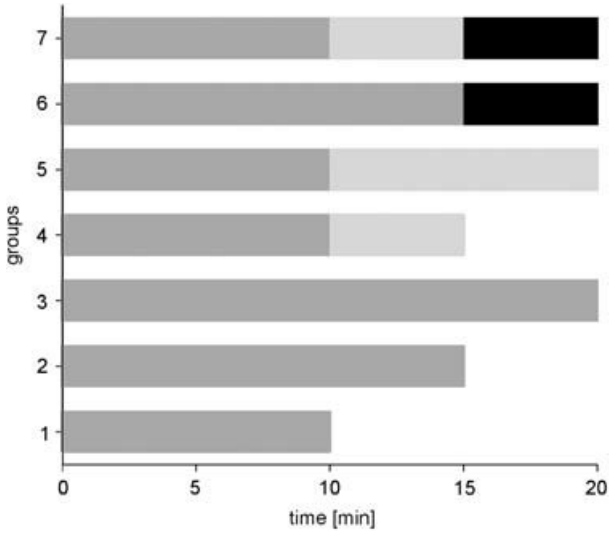

Figure 1.
Experimental protocol for the seven groups. Dark gray: perfusion with Krebs–Henseleit solution (KHS). Light gray: switch to perfusion with L*-bupivacaine 5 μg mL^{-1}. Black: infusion of insulin 10 mIU mL^{-1}.*

of 10 mIU mL^{-1}. At the end of each protocol, hearts were immediately freeze-clamped and stored at −60°C for further analysis.

Groups 1, 2 and 4 were used to determine adenine nucleotide changes over time. Groups 3 (Control), 5 (L-Bupi), 6 (Control Insulin) and 7 (L-Bupi Insulin) were compared regarding all parameters.

Local anaesthetics/Insulin

The commercially available local anaesthetic solution L-bupivacaine hydrochloride (Chirocain, Abbott GmbH & Co. KG, Wiesbaden, Germany) and insulin (B-Insulin, Berlin Chemie AG, Berlin, Germany) were used and diluted with NaCl 0.9%. We evaluated the dose-dependent effects of L-bupivacaine in three pilot experiments. A concentration of L-bupivacaine 5 μg mL^{-1} (15.4 μM) consistently led to myocardial depression (HR, +dP/d*t*, systolic pressure, flow >−30%).

Sample preparation

An organ sample was lyophilized with a Christ alpha 1-2-lyophilizator (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) and homogenized in perchloric acid 0.1 M. Precipitated proteins were separated by centrifugation and supernatant was neutralized with K_3PO_4 1 M. Adenine nucleotides were transformed to their respective 1,N^6-etheno-analogues with chloracetaldehyde to enable fluorescence detection. Neutralized sample 150 μL was mixed with KHS 2000 μL and citrate-phosphate buffer 770 μL was added. The reaction was started with chloracetaldehyde 80 μL. Samples were incubated for 40 min at 80°C and then instantly cooled to 4°C.

Chromatography

High-performance liquid chromatography for 1,N^6-etheno-analogues of adenine nucleotides was performed on a Waters Alliance 2690 (Waters Corp., Milford, Massachusetts, USA) coupled to a Merck-Hitachi (Hitachi, Tokyo, Japan) F 1050 fluorescence detector ($\lambda_{ex} = 280$ nm, $\lambda_{em} = 410$ nm) as previously reported [8]. In brief, separations were carried out on a Waters XTerra MS C18, 4.6 × 50 mm I.D. column, with a particle size of 5 μm and a 125 Å pore size. A guard column packed with the same sorbent was used to protect the analytical column. Samples (10 μL) were injected via an autosampler and compounds were eluted with a flow rate of 1.5 mL min^{-1} using a binary tetrabutylammoniumhydrogensulphate (TBSA)/acetonitrile gradient.

Statistical analysis

All data are presented as means ± standard deviations (SD). For between-group comparison, values from 0 to 10 min were averaged and compared with a 30 s average at 15 or 20 min, respectively. In pilot experiments, we found that insulin increased +dP/d*t* by approximately 80% in L-bupivacaine-induced cardiac depression. The mean coefficient of variation of the used data was 2% before and after insulin application, respectively, showing low within-subject variability. Power analysis yielded a sample size of three (an α of 0.05 and a β of 90%). We choose a sample size of five to further increase the power of our study.

All statistical analyses were carried out using SPSS software for MS Windows (Release 11.0; SPSS Inc., Chicago, IL, USA) using one-way ANOVA and Bonferroni's test. $P < 0.05$ was taken to indicate a statistical significance. All variables were tested for normal distribution with the Kolmogorov–Smirnoff test and were found to have normal distribution. Baseline values were compared using ANOVA and Bonferroni's test and showed no significant differences. All statistical analyses were performed with raw data.

Results

Baseline parameters for HR (307 ± 33 min^{-1}), coronary flow (12.7 ± 2.1 mL min^{-1}), PR (25 ± 1 ms), QRS (64 ± 6 ms), systolic pressure (80 ± 16 mmHg) and double product (23 930 ± 548 beats mmHg min^{-1}) showed no significant between-group differences. Perfusate oxygenation values and pH were

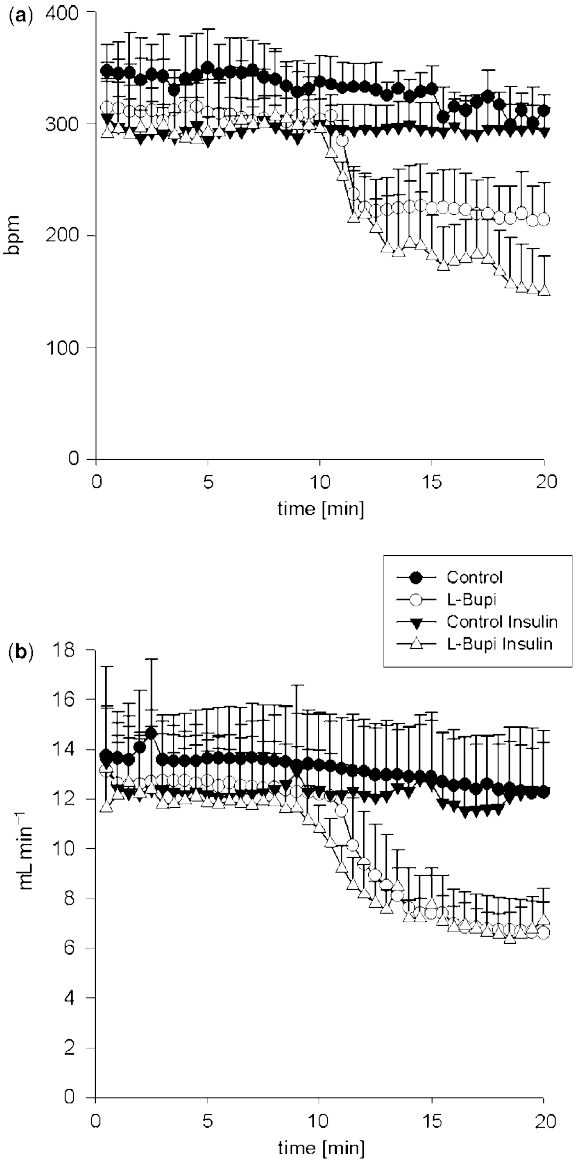

Figure 2.
*HR (beats min^{-1}, bpm ± SD) (**a**) and coronary flow (mL min^{-1} ± SD) (**b**) over the time course of the experiment for the four different groups* (n = 5 *per group): Control,* L-*Bupi (5 μg mL^{-1} at 10 min), Control Insulin (10 mIU mL^{-1} at 15 min) and* L-*Bupi Insulin (*L-*bupivacaine 5 μg mL^{-1} at 10 min and Insulin 10 mIU mL^{-1} at 15 min).*

comparable at baseline. The application of L-bupivacaine 5 μg mL^{-1} led to a significant decrease in MvO_2 (229 ± 41 in controls and 150 ± 62 μL min^{-1} g^{-1} in L-bupivacaine-treated hearts, $P < 0.05$), whereas ΔPO_2 remained stable (510 ± 66 vs. 465 ± 73 mmHg, not significant).

The application of L-bupivacaine 5 μg mL^{-1} 10 min into the experiment led to a significant decrease in HR (Fig. 2a, $P < 0.05$) and coronary flow (Fig. 2b, $P < 0.05$). Insulin application at 15 min had no effect on these parameters in controls

Table 1. Changes in percent at 15 min in relation to values at 10 min (100%) for the four different groups: Control, L-Bupi (5 μg mL^{-1} at 10 min), Control Insulin (10 mIU mL^{-1} at 15 min) and L-Bupi Insulin (L-bupivacaine 5 μg mL^{-1} at 10 min and Insulin 10 mIU mL^{-1} at 15 min).

	Control	L-Bupi	Control Insulin	L-Bupi Insulin
Heart rate	97 ± 6*,†	73 ± 8	100 ± 5*,†	64 ± 16
+dP/d*t*	96 ± 6*,†	62 ± 9	100 ± 7*,†	74 ± 19
Systolic pressure	93 ± 6*	68 ± 11	96 ± 4*	78 ± 17
Double product	92 ± 7*,†	48 ± 3	95 ± 7*,†	45 ± 12
Coronary flow	100 ± 9*	74 ± 20	100 ± 10†	67 ± 21
PR	99 ± 6*,†	164 ± 12	102 ± 2*,†	162 ± 16
QRS	101 ± 6*,†	143 ± 9	99 ± 4*,†	140 ± 22

ANOVA and *post hoc* Bonferroni's test.
*Significant vs. L-Bupi ($P < 0.05$); †significant vs. L-Bupi Insulin ($P < 0.05$).

Table 2. Changes (%) at 20 min in relation to values at 10 min (100%) for the four different groups: Control, L-Bupi (5 μg mL^{-1} at 10 min), Control Insulin (10 mIU mL^{-1} at 15 min) and L-Bupi Insulin (L-bupivacaine 5 μg mL^{-1} at 10 min and Insulin 10 mIU mL^{-1} at 15 min).

	Control	L-Bupi	Control Insulin	L-Bupi Insulin
Heart rate	92 ± 5*,†	69 ± 8	100 ± 5*,†	51 ± 12*
+dP/d*t*	97 ± 12*	59 ± 9	126 ± 27*	103 ± 10*
Systolic pressure	92 ± 8*	67 ± 11	113 ± 15*	106 ± 18*
Double product	86 ± 10*,†	44 ± 3	114 ± 12*,†,§	52 ± 14
Coronary flow	90 ± 4*,†	52 ± 4	100 ± 3*,†	60 ± 6
PR	100 ± 3*,†	162 ± 11	97 ± 5*,†	161 ± 21
QRS	104 ± 8*,†	143 ± 9	97 ± 4*,†	134 ± 21

ANOVA and *post hoc* Bonferroni's test.
*Significant vs. L-Bupi; †significant vs. L-Bupi Insulin; §significant vs. Control ($P < 0.05$).

and in L-bupivacaine-treated hearts. PR and QRS were increased by L-bupivacaine and they were also not influenced by insulin application. Double product was reduced by L-bupivacaine and was the only parameter to show a significant difference between Control and Control Insulin, and between Controls and hearts treated with L-Bupivacaine at 20 min (Tables 1 and 2 for an overview of changes and significances). +dP/d*t* (Fig. 3a, $P < 0.05$) and systolic pressure (Fig. 3b, $P < 0.05$) significantly decreased in L-bupivacaine-treated hearts. Insulin application led to a reversal of decreased +dP/d*t* and systolic pressure to baseline values within 5 min in the L-Bupi Insulin group. Similar positive inotropic effects were noted in the Control Insulin group.

The total adenine nucleotides (TAN = ATP + ADP + AMP + adenosine) in control animals over time were 3384 ± 739 at 10 min, 3027 ± 1115 at 15 min and 2855 ± 526 at 20 min of perfusion with KHS. L-Bupivacaine resulted in a TAN of

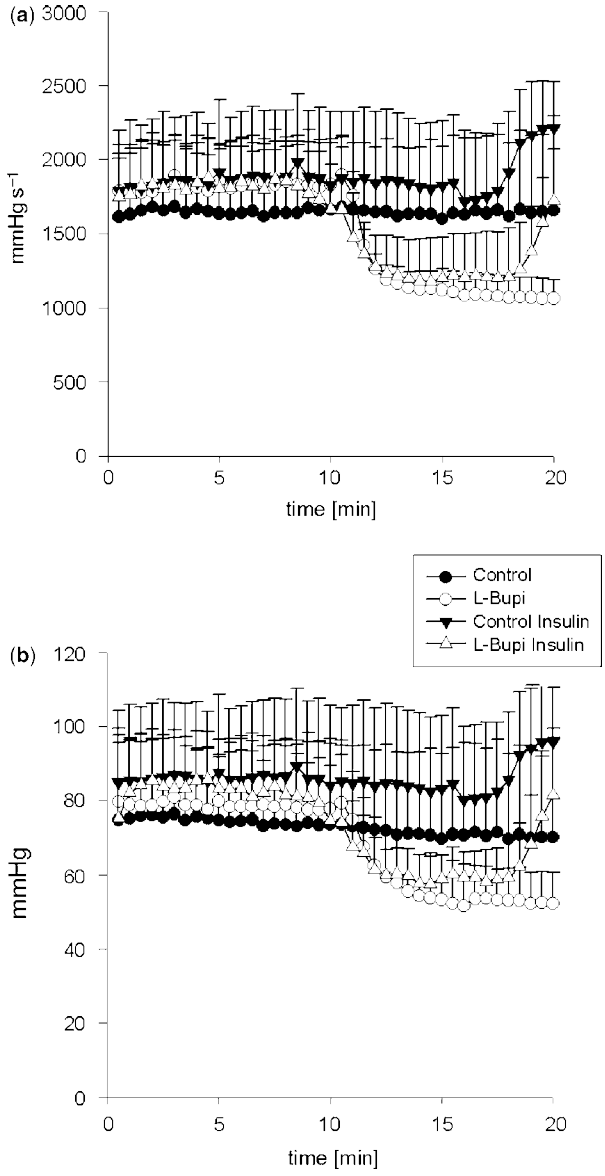

Figure 3.
*+dP/dt [mmHg s^{-1} ± SD] (**a**) and left ventricular systolic pressure [mmHg ± SD] (**b**) over the time course of the experiment for the four different groups* (n = 5 *per group): Control, L-Bupi (5 μg mL^{-1} at 10 min), Control Insulin (10 mIU mL^{-1} at 15 min) and L-Bupi Insulin (L-bupivacaine 5 μg mL^{-1} at 10 min and insulin 10 mIU mL^{-1} at 15 min).*

2874 ± 601 at 15 min and 2457 ± 486 at 20 min. Insulin application led to a TAN of 3357 ± 749 in Controls and of 3252 ± 487 in L-bupivacaine-treated hearts. All values are presented as nmol g^{-1} dry organ weight (Fig. 4) and none reached between-group significances.

Discussion

The isolated, perfused Langendorff rat heart preparation has limitations in the assessment of local

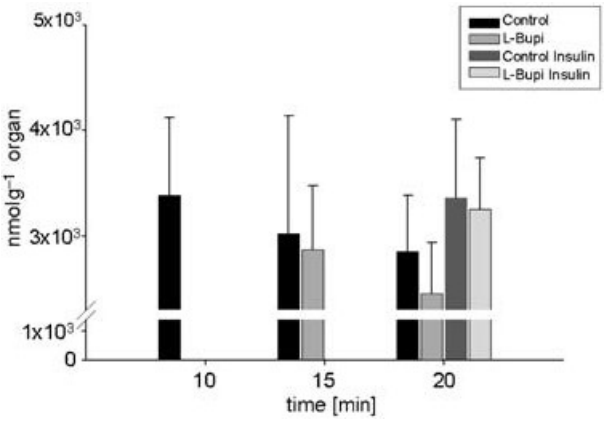

Figure 4.
Total adenine nucleotide pool (ATP + ADP + AMP + adenosine = TAN) [nmol g^{-1} organ ± SD] at 10, 15 and 20 min for Controls, L-Bupi (5 μg mL^{-1} at 10 min), Control Insulin (10 mIU mL^{-1} at 15 min) and L-Bupi Insulin (L-bupivacaine 5 μg mL^{-1} at 10 min and Insulin 10 mIU mL^{-1} at 15 min; n = 5 *per group).*

anaesthetic toxicity, e.g. hearts are denervated during the preparation process. In this model, spontaneous HR and coronary flow differs from the *in vivo* situation and no basal insulin was added to the KHS. On the other hand, the isolated heart has important advantages in assessing coronary and myocardial effects. The preparation avoids mechanical, humoral and autonomic nervous system influences. We chose a blood and protein-free perfusate to guarantee stable local anaesthetic perfusate concentrations. pH, electrolyte, glucose and perfusate gas concentrations can be maintained at a constant level. No continuous form of anaesthesia is necessary. Finally, the target concentration of local anaesthetics in the perfusate can easily be manipulated and/or kept constant. We chose TAN measurement as an indicator of myocardial energy state. Phosphocreatine as a marker for early energy depletion might have been more sensitive. In general, one also has to take into account a possible differential substance effect in different species. Therefore, conclusions concerning effects in human beings have to be drawn with caution.

Recently, two papers have shown striking *in vivo* effects of insulin–glucose–potassium infusion in bupivacaine-induced cardiovascular collapse [3,4]. No previous studies have been performed evaluating the effects of insulin application in L-bupivacaine-induced myocardial toxicity using the isolated heart model. L-Bupivacaine application led to an impairment of myocardial conduction, reflected by an increase in PR and QRS duration, an effect not reversed by insulin. An antagonistic effect

of insulin on the bupivacaine-induced outward potassium current (I_{to}) has been hypothesized because of *in vivo* HR recovery [3], an effect that we cannot confirm *in vitro*. L-Bupivacaine-treated hearts showed an impairment of contractility. Interestingly, this effect could be partially reversed by insulin. We could therefore document a direct positive inotropic action of insulin in bupivacaine-induced myocardial depression. Insulin is known to have a partial *in vivo* positive inotropic effect mediated by adrenal catecholamines [10]. Cho and colleagues did not evaluate this possibility [3]; using an isolated model we can exclude this indirect positive inotropic effect. As previously described by Burmester and colleagues, L-bupivacaine showed vasoconstrictive properties [11]. Although improved myocardial energetics have been considered to play a key role in the past [5], we found evidence for a clear positive inotropic insulin action, without significant changes in myocardial adenine nucleotides in L-bupivacaine-induced myocardial toxicity. However, power analysis of our study was based on $+dP/dt$ effects and it is therefore possible that our study was underpowered concerning differences in TAN. Performing uncorrected unpaired *t*-tests solely comparing data of the L-Bupi and Control Insulin and L-Bupi and L-Bupi Insulin groups at 20 min yield *P*-values of 0.054 and 0.033, respectively.

Our *in vitro* experimental protocol differs from the *in vivo* work on insulin effects and bupivacaine-induced cardiovascular depression by Kim [4] and Cho and colleagues [3] concerning continuous application of bupivacaine during insulin intervention. One major clinical approach to manage local anaesthetic toxicity is the application of catecholamines. In beginning myocardial depression epinephrine exerts positive ino-, chrono-, dromo- and bathmotropic effects. These effects counteract local anaesthetic-induced myocardial depression and also promote local anaesthetic redistribution and systemic clearance. The striking effects seen in prior work on insulin application in local anaesthetic toxicity have previously not been attributed to the positive inotropic effect we found by using an isolated heart preparation.

Previous work on the effects of insulin on local anaesthetic toxicity was conducted with racemic bupivacaine. R-bupivacaine application leads to a more pronounced delay in AV conduction [12], prolongation of PR and QRS intervals and occurrence of arrhythmia [13] compared to L-bupivacaine. We therefore chose the single enantiomer L-bupivacaine to be able to ignore possible differential effects resulting from the application of racemic bupivacaine. In human beings, concentrations of 1.5 [14]–2.6 [15] $\mu g\,mL^{-1}$ of racemic bupivacaine have been shown to elucidate symptoms of central nervous toxicity. In one study in human volunteers L-bupivacaine caused less negative inotropic effects than racemic bupivacaine at similar concentrations (2.62 vs. 2.25 $\mu g\,mL^{-1}$) [16]. We used a Krebs–Henseleit perfused isolated heart model which does not include plasma proteins. Therefore, all local anaesthetics are 'unbound'. The negative inotropic and chronotropic effects of L-bupivacaine were consistently observed at a concentration of 5 $\mu g\,mL^{-1}$ in our experiments; a detailed assessment of cellular mechanisms was not the aim of the study.

Positive inotropy has been found in the majority of studies on myocardial effects of insulin [17], but some authors documented no or even a negative myocardial effect [18]. Our results showed explicit positive inotropy. The exact mechanisms leading to this effect, however, have not yet been clearly defined. In isolated ventricular bands, the positive inotropic effects have been shown to be independent of the presence of glucose [19]. Insulin-stimulated glycolytic ATP production increases sarcoplasmatic-reticulum (SR)-associated calcium ATPase activity and thereby augments contractile function [20] potentially by directly binding to myocardial SR ATPase [21]. An influence on myocardial contractility has been described via a stimulation of the sarcolemmal Na^+/Ca^{2+} exchange [22,23]. In isolated guinea pig hearts, the effect of insulin is dose-dependent and influenced by the extracellular calcium concentration [24]. In that study, no positive inotropic effect of insulin was observed at the extracellular calcium concentration we used in our experiments (2.5 $mmol\,L^{-1}$). In this point our results are inconsistent. Changing extracellular calcium concentration has failed to be effective as a method of reversing myocardial negative inotropic action induced by local anaesthetics [25]. The negative inotropic effects of local anaesthetics have been mainly attributed to an inhibition of cAMP production [26], L-type calcium channels [27] and intracellular calcium effects [28].

In summary, insulin application in L-bupivacaine-induced cardiovascular depression and controls had a significant positive inotropic effect. This positive inotropic effect is not associated with a significant increase in adenine nucleotides. Electrophysiological parameters were not affected by insulin application. An indirect *in vivo* positive inotropic effect mediated by adrenal catecholamines can be excluded.

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