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Etomidate-anaesthesia, with and without fentanyl, compared with urethane-anaesthesia in the rat

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1 In spontaneously breathing rats, continuous infusion of etomidate with and without fentanyl caused a slight decrease in blood pressure and heart rate. Coadministration of fentanyl and etomidate in order to obtain full anaesthesia and analgesia resulted in respiratory depression.

2 In artificially ventilated rats both etomidate as well as the anaesthetic combination caused a strong reduction in aortic flow and an increase in total peripheral resistance. A single infusion of etomidate did not change blood pressure. Etomidate combined with fentanyl reduced blood pressure. Under adjusted ventilation blood pressure, aortic flow, max(dF/dt) and heart rate were progressively reduced during a 4 h period.

3 In contrast, urethane anaesthesia reduced aortic flow to a minor extent. Total peripheral resistance and max(dF/dt) were hardly affected. The slightly reduced blood pressure and blood gas variables remained stable during the experiment.

4 From pharmacokinetic studies it was established that effective etomidate plasma levels were maintained constant during the experimental period. Pharmacokinetic interaction between etomidate and fentanyl did not occur.

5 It is concluded that for anaesthesia of longer duration during cardiovascular experiments in rats, urethane is preferable to etomidate/fentanyl because it does not cause serious changes in basal haemodynamic variables.

Introduction

It is well known that anaesthetics can have significant effects upon haemodynamic variables in rats (Salgado & Krieger, 1976; Smith & Hutchins, 1979; 1980) which can lead to difficulties in interpreting actions of drugs upon the cardiovascular system. However, in many experiments anaesthesia is required and necessary. In order to exclude any interference between the substances to be tested and the anaesthetic, the effects of a drug should be investigated under at least two types of anaesthesia. Furthermore, it is desirable that the initial haemodynamic status in anaesthetized rats does not differ to a great extent from that in conscious animals. Therefore one has to look for an anaesthetic which influences the cardiovascular system as little as possible. The same holds true for Pa_{CO_2}, Pa_{O_2} and pH, since there is a relation between these variables and the circulation.

Urethane is frequently used as an anaesthetic for rats, mostly because it induces only minor changes in arterial blood pressure and respiration, has a wide margin of safety and gives a stable, long-lasting anaesthesia (Zandberg & Sangster, 1980).

Etomidate (**R**-(+)-ethyl-1(phenylethyl)1 H-imidazole-5-carboxylate sulphate) is an intravenous and potent, short-acting non-barbiturate hypnotic (Janssen, Niemegeers & Marsboom, 1975). From earlier investigations in man and dog (Jageneau, Xhonneux & Reneman, 1973; Doenicke, Gabanyi, Lemcke & Schurk-Bullich, 1974; Weymar, Eigenheer, Gethmann, Reinecke, Patschke, Tarnow & Brücker, 1974) etomidate anaesthesia seemed promising with regard to cardiovascular stability. For anaesthesia of longer duration it has to be given as a continuous infusion. Recently etomidate has been introduced as an adequate anaesthetic in total intravenous anaesthesia in man (Kay, 1977; Booij, Rutten & Crul, 1978; van Dijk, 1979). These properties of etomidate indicate that it might be a useful anaesthetic in cardiovascular research in the rat. Detailed haemodynamic studies following continuous administration of etomidate in rats are scarce. The present study was undertaken to examine the effects of appropriate anaesthetic doses of etomidate upon haemodynamic stability and blood gas variables

compared with urethane. Being a purely hypnotic agent, etomidate lacks analgesic properties. Therefore it is also necessary to consider the haemodynamic profile and effects upon blood gas variables following infusion of a combination of etomidate and fentanyl.

Since etomidate will be used as hypnotic for the entire duration of anaesthesia it is important to maintain plasma concentrations in steady-state following infusion of the drug, in order to obtain a balanced anaesthesia. Therefore, plasma levels of etomidate were also determined.

Methods

Male rats of an inbred Wistar strain (Wi/CPB TNO, Zeist, The Netherlands) weighing 250–300 g were used.

Etomidate with and without fentanyl in spontaneously breathing rats

Arterial blood pressure of conscious or anaesthetized spontaneously breathing rats was recorded from a carotid artery cannulated under ether anaesthesia 24 h before the experiment. Heart rate was derived from the blood pressure trace by a cardiotachometer (Biotachometer BT-1200, Narco Biosystems Inc.). Intravenously administered anaesthetics were infused via a jugular vein catheter introduced under ether anaesthesia 24 h before the experiment. Bolus infusion of the anaesthetics were given slowly over a period of 2–3 min, guided by arterial blood pressure in order to minimize initial cardiovascular responses. Blood gases and pH levels were monitored from arterial blood samples (100 μl) by means of a blood gas analyser (Instrumentation Laboratory Type 413) before each anaesthetic infusion and at regular time intervals following anaesthesia. Body temperature was maintained at 37.0 ± 0.5°C by radiant heat.

Urethane and etomidate with and without fentanyl in artificially ventilated rats

Pentobarbitone (60 mg kg^{-1}, i.p.) provided surgical anaesthesia for the implantation procedures. Artificial ventilation was carried out during the surgical period with a Harvard Model 680 small-animal respirator.

Ascending aortic flow (= cardiac output minus coronary flow) was measured by electromagnetic flowmetry according to the method of Smith & Hutchins (1979). An electromagnetic, precalibrated flowprobe (flowsensor for chronic applications, manufactured by Skalar Medical, Delft, The Netherlands) with an internal diameter of 2.1 mm was placed around the ascending aorta. The connector end of the probe-cable was exteriorized and sutured on the head. Following appropriate chest-closure, catheters (PE_{50}) were inserted into the aortic arch via the left common carotid artery and into the right jugular vein, and were exteriorized on the dorsal surface of the neck. The animals were allowed to recover for at least 4 days following implantation.

Aortic flow was measured continuously with a sine-wave calibrated electromagnetic flowmeter (Transflow 601-system, MDL 500, Skalar Medical, Delft, The Netherlands). Both pulsatile and mean aortic flow signals were then displayed on a Hewlett Packard recorder (HP 7758). Zero-flow reference was taken to be the diastolic level of the instantaneous aortic blood flow signal. Cardiac output was considered to be equal to aortic flow. The maximal acceleration of aortic blood flow (max(dF/dt)) was measured by differentiating the pulsatile flow signal by means of an analogue device (HP 8814 A derivative computer), set at an upper cut-off frequency of 100 Hz. The differentiated signal was displayed on the Hewlett Packard recorder. From this parameter and the peak of the phasic aorta flow, the ratio of maximal aortic acceleration to peak flow (max(dF/dt):peak flow) was calculated.

Blood pressure was recorded continuously from the aorta via a pressure transducer (HP type 1280C) connected to a Hewlett Packard recorder. Heart rate was derived from the pulse wave of the aortic flow. Total peripheral resistance was calculated from mean arterial pressure (MAP) and cardiac output.

The protocol for the experiment was as follows. Recordings were made at the same time of day, between 09 h 00 min and 13 h 00 min 4–7 days following the implantation procedures. At that time blood samples were drawn from the arterial catheter in conscious animals. Blood samples were analysed by means of a blood gas analyser 413 (Instrumentation Laboratory) for $PaCO_2$, PaO_2, pH and standard HCO_3^-. Furthermore, blood pressure and the above mentioned flowmetric variables were recorded in the conscious rats 5–10 min before administration of the anaesthetic. After sufficient anaesthesia the trachea was cannulated and artificial ventilation was applied. Tidal volume and pump frequency were adjusted in such a way that $PaCO_2$ and PaO_2 values were comparable with those obtained in unrestrained conscious animals as measured in a separate experiment (Table 1). Haemodynamic and blood gas values were determined at regular intervals after the start of the infusion of the anaesthetic or in the case of urethane, after a 30 min stabilization period. The experimental period was 4 h. Throughout the experiments saline (0.9% w/v NaCl in distilled water) was infused via a cannulated femoral vein in a volume of 2.25 ml for 1 h and rectal temperature was controlled and kept

Table 1 Blood gas values in conscious animals measured several days following cannulation of a carotid artery

Day	Pa_{CO_2}	Pa_{CO_2}	*pH*	HCO_3^-	CO_2	*SB*	*Ht*
1	31.1 ± 1.1	95.5 ± 4.2	7.488 ± 0.008	24.0 ± 0.8	25.1 ± 0.8	26.8 ± 0.6	0.49 ± 0.02
2	33.3 ± 0.8	87.5 ± 1.3	7.471 ± 0.014	23.8 ± 0.5	24.8 ± 0.6	26.3 ± 0.6	0.48 ± 0.02
4	33.8 ± 0.9	93.6 ± 4.0	7.473 ± 0.015	24.2 ± 0.6	25.3 ± 0.6	26.5 ± 0.6	0.46 ± 0.02

Mean values ± s.e.mean ($n = 7$).
Pa_{CO_2} = arterial CO_2 tension; Pa_{O_2} = arterial O_2 tension; SB = standard bicarbonate; Ht = hematocrit.

constant at approximately 37.0 ± 0.5°C by radiant heat.

Determination of etomidate in plasma

In a separate study, plasma levels of etomidate were determined both following intravenous infusion of etomidate and after infusion of a combination of etomidate with fentanyl in spontaneously breathing rats. Blood samples were collected from the carotid artery, cannulated 24 h prior to the experiment, at various time intervals following administration of the anaesthetic.

Determination of etomidate in plasma was carried out according to Haring, Dijkhuis & van Dijk (1980). Blood samples in cooled (4°C) polyethylene centrifuge tubes, immediately centrifuged at approximately 1000 *g* and the pipetted plasma was frozen (−20°C) as soon as possible to prevent hydrolysis of etomidate.

A volume of 0.1 ml of plasma was pipetted into a cold glass-stoppered tube; 0.1 ml internal standard solution was added (propoxate HCl, 5 mg/l aqueous solution) and 5 ml solvent (hexane/diethylether, 9:1, v/v). After gentle shaking for 10 min the samples were centrifuged for 5 min at approximately 1000 *g*. The organic layer was transferred to another tube and evaporated at 40°C in a stream of nitrogen. The residue was dissolved in 50 µl acetone-methanol (8:2, v/v).

For the g.l.c. determination a Sigma 3 gas chromatograph (Perkin-Elmer) was used, equipped with a nitrogen/phosphorus detector (bead current was set at 50% of full scale at attenuation 8). The stationary phase was OV 17, 3%, on Gas Chrom Q (80–100 mesh) packed into a 1.5 m glass column (inner diameter 3 mm). The carrier gas (nitrogen) flow rate was 22 ml/min. Temperature settings were as follows: oven, 215°C; injection port, 265°C; detector, 260°C. From the solution of the extraction residue 1 or 2 µl samples were injected. Under the conditions described, the retention times were etomidate 2.2 min and propoxate 3.2 min. The calibration curve of the etomidate concentrations versus peak hight ratio etomidate/propoxate was linear in the range of 0.05–2.0 mg l^{-1} ($r = 0.9975$). The lower limit of detection in plasma was 0.1 mg l^{-1}, the recovery from plasma was 100 ± 6% (mean ± s.d., $n = 12$).

Materials and statistics

Drugs used in this study were: etomidate sulphate (kindly supplied by Janssen Pharmaceutica, Beerse, Belgium), etomidate concentrations are expressed in terms of their free base, urethane (Brocacef, Maarssen, The Netherlands), fentanyl (Janssen Phar-

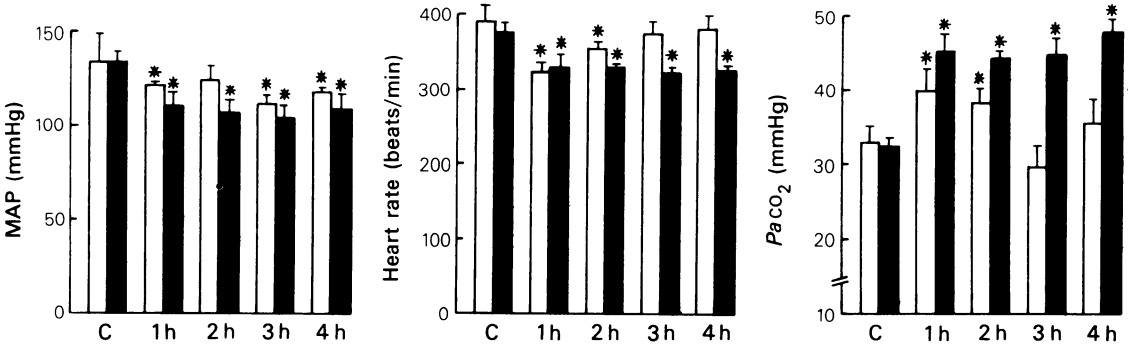

Figure 1 The influence of etomidate (open columns) and a combination of etomidate with fentanyl (closed columns) upon blood pressure, heart rate and Pa_{CO_2} in spontaneously breathing rats ($\bar{x} \pm$ s.e.mean, $n = 4$) at regular time intervals following start of infusion. C denotes pre-anaesthetic values in conscious animals. MAP = mean arterial pressure.
* significantly different from C ($P < 0.05$).

maceutica, Beerse, Belgium), propoxate HCl (R 7464) (kindly supplied by Janssen Pharmaceutica, Beerse, Belgium), hexane (Merck, Darmstadt, GFR), diethylether (Merck, Darmstadt, GFR), acetone (Merck, Darmstadt, GFR) and methanol (Merck, Darmstadt, GFR) were also used.

All results are expressed as mean ± s.e.mean and were analysed by Student's t test with $P < 0.05$ considered significant.

Results

Effects of etomidate with and without fentanyl upon cardiovascular variables and blood gases in spontaneously breathing rats

Effects of etomidate Intravenous administration of etomidate (bolus infusion 5 mg kg^{-1} followed by continuous infusion of 10 mg kg^{-1} h^{-1}) produced initially a decrease in arterial blood pressure from 134 ± 15 mmHg to 83 ± 16 mmHg ($n = 4$). Within 20 min MAP had returned to 122 ± 2 mmHg ($P < 0.05$). During a period of 4 h, blood pressure remained at a constant level slightly below values as measured before the administration of the anaesthetic. Cardiac frequency was initially strongly reduced from 390 ± 23 beats min^{-1} to 284 ± 33 beats min^{-1} ($P < 0.05$) and after 30 min almost returned to control values (Figure 1).

Etomidate caused an elevation of Pa_{CO_2} from 33.3 ± 0.8 mmHg up to 39.8 ± 2.6 after 1 h. During the 4-h period Pa_{CO_2} tended to return slowly to the initial control value (Figure 1). Pa_{O_2} remained within a range of 80–90 mmHg. pH was slightly reduced ($P < 0.05$) and returned almost to the initial value. Standard bicarbonate did not change significantly (Table 2).

After administration of etomidate full and adequate hypnosis was present although analgesia was insufficient.

Effects of etomidate in combination with fentanyl From preliminary studies it was derived that intravenous infusion of a combination of etomidate (bolus 5 mg kg^{-1}; continuous infusion 10 mg kg^{-1} h^{-1}) and fentanyl (bolus 2.5 μg kg^{-1}; continuous infusion 15 μg kg^{-1} h^{-1}) resulted in adequate anaesthesia and analgesia. Initially this combination resulted in a small decrease in MAP from 134 ± 5 mmHg to 111 ± 6 mmHg ($n = 4$) which remained throughout the experiment (Figure 1). Heart rate was initially strongly reduced from 375 ± 15 beats min^{-1} to 280 ± 12 beats min^{-1} ($P < 0.05$) after 30 min. After 1 h, heart rate was 328 ± 16 and remained at the same level throughout the experiment (Figure 1). This anaesthetic mixture caused a relatively strong elevation of Pa_{CO_2} from 32.3 ± 0.9 mmHg up to 45.1 ± 2.0 mmHg after 1 h. During the measuring period, Pa_{CO_2} continued to be rather high in comparison to pre-anaesthetic values.

Table 2 The influence of etomidate (E) and a combination of etomidate with fentanyl (E/F) upon pH and standard bicarbonate (SB)

			Conscious	*1 h*	*2 h*	*3 h*	*4 h*
Spontaneous respiration	E	pH	7.470 ± 0.014	7.396 ± 0.024	7.442 ± 0.018	7.444 ± 0.019	7.460 ± 0.027
		SB	26.5 ± 0.8	25.5 ± 0.4	26.4 ± 0.4	23.8 ± 0.8	26.2 ± 0.6
	E/F	pH	7.525 ± 0.007	7.372 ± 0.014	7.369 ± 0.002	7.360 ± 0.013	7.328 ± 0.010
		SB	28.0 ± 0.3	25.3 ± 0.2	24.8 ± 0.2	24.4 ± 0.3	23.8 ± 0.4
Artificial ventilation	E	pH	7.463 ± 0.016	7.520 ± 0.028	7.531 ± 0.018	7.561 ± 0.007	7.560 ± 0.008
		SB	23.7 ± 0.7	23.7 ± 0.6	23.0 ± 0.9	25.0 ± 0.6	23.1 ± 0.6
	E/F_I	pH	7.503 ± 0.019	7.529 ± 0.030	7.523 ± 0.058	7.516 ± 0.017	7.528 ± 0.020
		SB	28.1 ± 1.1	25.2 ± 1.4	23.6 ± 1.0	23.9 ± 0.5	24.2 ± 0.7
	E/F_{II}	pH	7.502 ± 0.012	7.457 ± 0.012	7.417 ± 0.023	7.356 ± 0.020	7.332 ± 0.023
		SB	27.8 ± 1.1	24.7 ± 1.1	23.3 ± 1.0	20.2 ± 1.5	18.9 ± 1.6

I Ventilatory regimen: 70 strokes min^{-1}, tidal volume 3 ml, FiO_2 0.20
II Ventilatory regimen: 45 strokes min^{-1}, tidal volume 3 ml, FiO_2 0.25
Mean values ± s.e.mean of 4 rats with spontaneous respiration and 5 rats with artificial ventilation.

Pa_{O_2} fluctuated within the normal range. Arterial pH and standard bicarbonate were considerably reduced ($P < 0.05$) in comparison with pre-anaesthetic levels (Table 2).

Effects of etomidate with and without fentanyl and urethane upon haemodynamics and blood gases in artificially ventilated rats

Effects of urethane In order to obtain Pa_{CO_2} and Pa_{O_2} values comparable to those obtained in unrestrained conscious rats (Table 1), the ventilation pump was set at 60 strokes min^{-1} and a stroke volume of 3 ml, FiO_2 being 0.25.

Urethane (1 g kg^{-1}, i.p., $n = 5$) reduced blood pressure from a pre-anaesthetic level of 119 ± 6 mmHg to 97 ± 5 mmHg at 30 min after injection. During the experiment, blood pressure appeared to increase slightly to about 90% of pre-anaesthetic control value (Figure 2). Cardiac frequency was slightly but not significantly increased. Aortic flow was reduced significantly ($P < 0.05$) from 87 ± 6 ml min^{-1} to 66 ± 4 ml min^{-1} 30 min after injection and remained throughout the experiment at a level of about 69 ml min^{-1}. The contractile indices max(dF/dt) and the ratio max(dF/dt):peak flow were hardly influenced. Total peripheral resistance was not changed significantly. Throughout the experiment, no significant changes in blood gases (Figure 2) and pH values occurred.

Effects of etomidate From preliminary studies a ventilation regimen of 70 strokes min^{-1} and a tidal volume of 3 ml (FiO_2 being 0.20) was established for etomidate anaesthetized rats in order to achieve blood gas values similar to those in conscious rats. Etomidate induced initially a decrease in blood pressure. After 30 min no statistically different blood pressure (127 ± 7 mmHg) was observed in comparison with pre-anaesthetic values (118 ± 9 mmHg). Two hours after injection of etomidate, blood pressure was slightly but not significantly reduced from control (Figure 2). Cardiac frequency was not influenced. Aortic flow was strongly depressed by etomidate. After 1 h this variable declined from 76 ± 2 to 42 ± 4 ml min^{-1} (Figure 2). At the end of the 4-h period, aortic flow was reduced to 35 ± 2 ml min^{-1}. The contractile index max(dF/dt) was also strongly depressed by etomidate. During the experiment this parameter gradually decreased from a pre-anaesthetic level of 649 ± 40 ml s^{-2} to 373 ± 43 ml s^{-2} (Figure 2). The ratio max(dF/dt):peak flow was hardly influenced by etomidate. Etomidate produced a marked increase in total peripheral resistance (Figure 2). The mean pre-anaesthetic values for Pa_{CO_2} were not significantly different from those obtained 1 h following infusion of etomidate. However, in all rats Pa_{CO_2} was reduced at 2, 3 and 4 h after infusion (Figure 2). pH was slightly increased after administration of etomidate. Standard bicarbonate was not influenced (Table 2).

Effect of etomidate in combination with fentanyl Haemodynamic alterations produced by the combination of etomidate and fentanyl were examined under two different ventilation regimens. In a first series (I) of experiments ($n = 5$) rats were ventilated with 70 strokes min^{-1} and a tidal volume of 3 ml (FiO_2 being 0.20). However at 1 h following application of the anaesthetic mixture, Pa_{CO_2} (23.8 ± 1.2) was significantly ($P < 0.05$) different from control (32.5 ± 1.4) (Figure 2).

Therefore a second series (II) of experiments ($n = 5$) was performed in which ventilation was adjusted to 45 strokes min^{-1} and a stroke volume of 3 ml (FiO_2 being 0.25). Throughout this experiment Pa_{CO_2} and Pa_{O_2} ranged between 32.8 ± 1.6 and 33.1 ± 1.4 mmHg respectively 81 ± 5 mmHg and 91 ± 8 mmHg, values which were comparable to those obtained in conscious animals. In animals of this group pH and standard bicarbonate gradually declined (Table 2).

In both series of experiments the anaesthetic combination reduced aortic flow from 75 ± 1 ml min^{-1} to 40 ± 3 ml min^{-1} (I) respectively 81 ± 6 ml min^{-1} to 40 ± 4 ml min (II) after 30 min. During the course of the experiment, aortic flow decreased to 33 ± 2 ml min^{-1} (I) and 27 ± 6 ml min^{-1} (II) (Figure 2).

Blood pressure responses to the anaesthetic combination in both groups (I and II) were not consistent. In group I, in 2 out of 5 rats blood pressure was not decreased after 30 min but was even slightly higher compared with pre-anaesthetic values. During the experiment, blood pressure decreased gradually. The other 3 animals responded immediately with a fall in blood pressure. At the end of the experiment blood pressure had fallen from 119 ± 9 mmHg to 83 ± 6 mmHg. In group II, in 2 out of 5 rats blood pressure was not seriously affected. For the other 3 rats, a distinct fall in blood pressure was seen at 1 h following infusion. At the end of the experiment, blood pressure had decreased from 106 ± 7 mmHg ($n = 5$) to 55 ± 13 mmHg ($n = 5$).

In group I, heart rate was slightly raised during the experiment. In group II, heart rate was significantly ($P < 0.05$) reduced from 400 ± 19 beats min^{-1} to 330 ± 19 beats min^{-1}. For both groups the contractile index max(dF/dt) was significantly reduced ($P < 0.05$). In group I, the ratio max(dF/dt):peak flow was significantly depressed from 119 ± 7 s to 105 ± 5 s and in group II a decline from 127 ± 4 to 83 ± 12 s was seen. TPR was significantly increased in both groups by the anaesthetic combination.

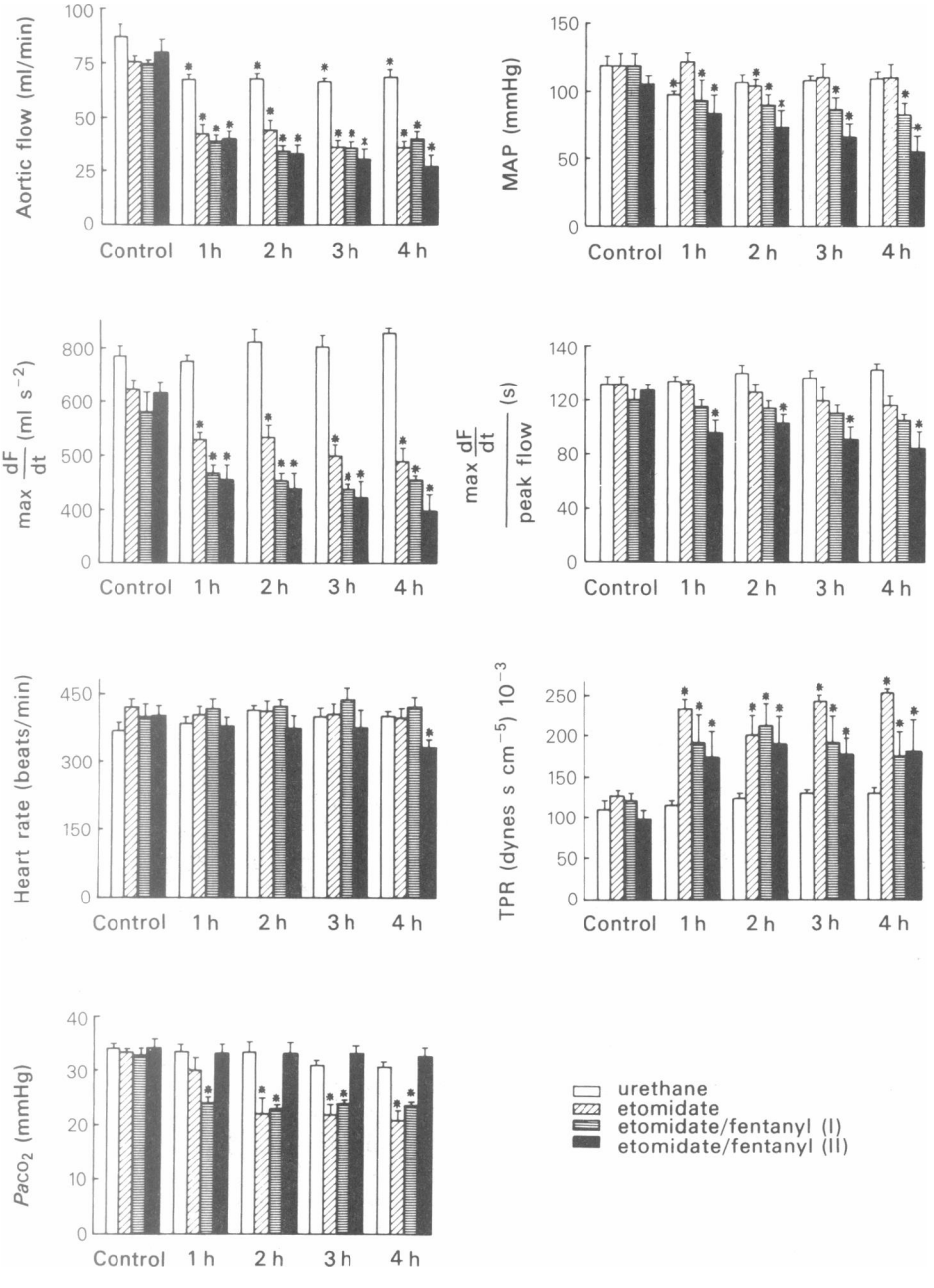

Figure 2 The influence of urethane ($1\,g\,kg^{-1}$, i.p.) and etomidate (priming dose $5\,mg\,kg^{-1}$, infusion rate $10\,mg\,kg^{-1}\,h^{-1}$) or a combination of etomidate with fentanyl (priming dose $5\,mg\,kg^{-1}$ respectively $2.5\,\mu g\,kg^{-1}$, infusion rate $10\,mg\,kg^{-1}\,h^{-1}$ respectively $15\,\mu g\,kg^{-1}\,h^{-1}$) upon different cardiovascular parameters and Pa_{CO_2} in artificially ventilated rats ($x \pm$ s.e.mean, $n = 5$) at regular time intervals following start of infusion or in case of urethane after 30 min stabilization. Control corresponds with pre-anaesthetic values in conscious animals. MAP = mean arterial pressure; dF/dt = first derivative of aortic flow; TPR = total peripheral resistance.
* significantly different from control ($P < 0.05$).

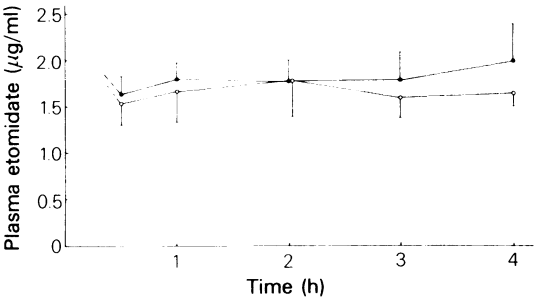

Figure 3 Mean (±s.d., $n = 6$) plasma etomidate concentrations following administration of etomidate (priming dose 5 mg kg^{-1}, infusion rate 10 mg kg^{-1} h^{-1}, ●) or etomidate combined with fentanyl (priming dose 5 mg kg^{-1} and 2.5 μg kg^{-1} respectively, infusion rate 10 mg kg^{-1} h^{-1} and 15 μg kg^{-1} h^{-1}, respectively, ○).

Plasma levels of etomidate following infusion of etomidate and a combined infusion of etomidate with fentanyl

Initially, single dose experiments were carried out with intravenous doses of 5 and 10 mg kg^{-1} to calculate the parameters (priming dose and infusion rate) for the intravenous infusion. The sensitivity of the method did not allow us to detect the third compartment mentioned by Lewi, Heykants & Janssen (1976) so a two-compartment analysis was made. The apparent volume of distribution (V_β) found was 1.55 h^{-1}. Aiming at a steady-state concentration of 1.0–1.5 mg l^{-1} an infusion rate (A) of 12 mg kg^{-1} h^{-1} was chosen, with a priming dose (D*) of 7 mg kg^{-1}.

The plasma levels found in practice with this regimen were too high, with a tendency to increase after 2 h. When the infusion rate was lowered to 10 mg kg^{-1} h^{-1} and the priming dose to 5 mg kg^{-1} steady-state levels of about 1.7 mg kg^{-1} were obtained. Using this regimen the potential influence of coadministration of fentanyl (infusion rate: 15 μg kg^{-1} h^{-1}; priming dose 2.5 μg kg^{-1}) was investigated. The results are shown in Figure 3.

Discussion

In the present study the effects of etomidate with and without fentanyl were compared with those obtained following urethane anaesthesia and were evaluated by analysis of haemodynamics, blood gases and plasma levels.

In spontaneously breathing rats, the combination of etomidate and fentanyl produced a small but significant ($P < 0.05$) decrease in blood pressure and cardiac frequency. Whether this decrease in blood pressure and heart rate is substantial is not obvious. Since initial blood pressure values were relatively high it is probable that the rats were stressed. Therefore a decrease in blood pressure might be attributed to unstressing of the animals by the anaesthetic.

From Pa_{CO_2} values it is obvious that this anaesthetic mixture induces a distinct respiratory depression. The initial decrease in pH is the consequence of this depression. Since standard bicarbonate showed a downward course during the experimental period the decrease in pH might be partially of metabolic origin. The results as depicted in Figure 1 indicate that a great part of the observed cardiovascular and respiratory effects can be attributed to fentanyl (Gardocki & Yelnosky, 1964; Hug and Murphy, 1979; Laubie, Schmitt & Drouillat, 1977). However, the possibility cannot be excluded that the decrease in pH and standard bicarbonate is a result of the combination of etomidate with fentanyl.

In cardiovascular experiments artificial ventilation is often preferred because it is easy to control and to keep biochemical parameters within normal ranges. This preference is enhanced by the fact that most anaesthetics depress respiration. From Figure 2 it can be observed that etomidate alone does not influence blood pressure seriously, possibly as a consequence of an increased total peripheral resistance, since cardiac output was decreased to a great extent. It is not clear whether the change in peripheral resistance is caused by direct influences upon the peripheral circulation itself or if it is caused by compensatory mechanisms due to a strong depression of myocardial function. Since no significant changes in cardiac frequency occur, the latter possibility seems less reasonable. Moreover, if the ratio max(dF/dt):peak flow is a reliable parameter for intrinsic myocardial contractility, independently from preload and afterload (Nutter, Noble & Hurst, 1970), a direct cardiodepressive action by this anaesthetic can be ruled out. Therefore one can speculate that a primary increase in peripheral resistance might lead to a secondary decrease in pumping function. The underlying mechanism for the increased resistance is not yet known.

In group I the anaesthetic combination of etomidate/fentanyl reduced arterial pressure significantly more than after etomidate infusion alone, which might be explained by a less strong increase in total peripheral resistance (Figure 2). Since Pa_{CO_2} values were different from those obtained in unanaesthetized animals, ventilation was adjusted (group II) in such a way that blood gas values were comparable to those in conscious animals. Now, it was seen that the cardiovascular system was more disturbed. Most of the circulatory parameters (MAP, aortic flow, max(dF/dt) etc.) were decreased to a greater extent than in group I. It was observed that with adjusted ventilation conditions (group II), a

gradual decrease in standard bicarbonate was accompanied with a concomitantly reduced pH and constant Pa_{CO_2}, indicating a metabolic origin of the change in pH. The observed acidosis might well explain the reduction in arterial pressure during the experimental period probably by embarrassment of myocardial function as can be established from the reduction in cardiac output and the ratio max(dF/dt):peak flow during the experiment. It appears that in group I this acidosis and therefore the cardiovascular response to the anaesthetic mixture is counteracted by the lower Pa_{CO_2} values.

As opposed to the results found with etomidate/fentanyl anaesthesia, changes in haemodynamic variables and blood gases following urethane anaesthesia are generally small or absent when compared to reference values from conscious animals. Moreover, from the results it can be concluded that all measured variables remained stable during the experimental period.

If etomidate is used as a hypnotic for the entire duration of anaesthesia, effective plasma levels should be kept constant without unwanted accumulation of drug, since otherwise pharmacokinetic or pharmacological interaction between the anaesthetic and the drug to be tested may confuse the interpretation of results. From the results it can be assumed that plasma levels were constant during the experimental period. Furthermore, pharmacokinetic interaction between etomidate and fentanyl did not seem to occur.

From the results it might be concluded that because of the marked effects of etomidate and fentanyl on the cardiovascular system, this combination is not suitable for cardiovascular research in rats. From our studies it appeared that much higher doses of etomidate were required for adequate sleep duration and hypnosis in rat ($2-5\ mg\ kg^{-1}$) than in man ($0.3\ mg\ kg^{-1}$) and dog ($0.8-2.5\ mg\ kg^{-1}$). The relatively higher doses might explain the occurrence of more pronounced cardiovascular side effects in rat than in other species. In contrast, urethane appears to be a suitable anaesthetic because it does not introduce significant changes in basal haemodynamic variables.

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