Sufentanil and medetomidine anaesthesia in the rat and its reversal with atipamezole and butorphanol

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Summary

Injectable anaesthetics are widely used to anaesthetize rats, but recovery times are often prolonged. Reversible anaesthetic regimens have the advantage that animals may be recovered quickly, thus reducing the incidence of postoperative complications such as hypothermia, and also providing a means of treating inadvertent anaesthetic overdose. This study assessed and compared the characteristics of anaesthesia induced with combinations of sufentanil and medetomidine administered as a single subcutaneous or intraperitoneal dose, and reversal with butorphanol and atipamezole. Combinations of sufentanil/medetomidine at 40 μg/150 μg and 50 μg/150 μg/kg administered subcutaneously, and 80 μg/300 μg/kg by intraperitoneal injection were found to produce surgical anaesthesia for 101 ± 49, 124 ± 45 and 76 ± 23 min (means ± SD) respectively. All three combinations produced marked respiratory depression 30 min after injection (< 50% of resting respiratory rate). Oxygen saturation, measured by pulse oximetry, was < 50% in all groups 30 min following drug administration. Subcutaneous administration is recommended since it resulted in a more reliable and more rapid induction of anaesthesia than intraperitoneal administration. The administration of butorphanol and atipamezole (0.2/0.5 mg/kg s.c.) resulted in a rapid (< 7 min) reversal of anaesthesia and an associated respiratory depression. The induction of anaesthesia with sufentanil/medetomidine and its reversal with a combination of atipamezole and butorphanol is an effective technique for anaesthetizing rats. However, due to the marked respiratory depression and the resulting hypoxia, we recommend that this regimen should only be used in animals which are free from respiratory disease and that oxygen should be provided during anaesthesia.

Keywords Rat; anaesthesia; sufentanil; medetomidine; atipamezole; butorphanol

In most instances injectable anaesthetics are administered by the intraperitoneal, subcutaneous or intramuscular routes. This, however, does not allow an adjustment of the anaesthetic dose to meet the requirements of the individual animal, and the relatively high dose rates required, in comparison to those associated with intravenous administration, result in prolonged recovery times (Flecknell 1996). These problems can be reduced if the anaesthetic regimen can be completely or partially reversed using specific antagonist drugs. A number of different combinations have been described, for example partial reversal of fentanyl/fluanisone (Flecknell *et al.* 1989), ketamine/xylazine (Lipman *et al.* 1987), and ketamine/medetomidine (Morris 1991). The effects of combinations of fentanyl, a μ-agonist opioid, and medetomidine, an α-adrenoceptor agonist, have been

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described in the rat (Hu *et al.* 1992). This combination, however, has the disadvantages of requiring a relatively large volume of injectate (1 ml/100 g i.p.) and it was reported that subjects developed severe respiratory depression. Sufentanil, another μ-agonist opioid, has been reported to be 16 times more potent in rats than fentanyl, and reported to provide safe and effective anaesthesia (Niemegeers *et al.* 1976). When used alone, potent μ-opioids produce marked skeletal muscle rigidity, and are not generally considered acceptable within anaesthetic regimens. When combined with sedatives or tranquillizers, balanced anaesthesia can be produced. This study investigates the effects of sufentanil in combination with medetomidine and its reversal with butorphanol and atipamezole in rats.

The intraperitoneal administration of opioids results in a high first-pass liver metabolism and subcutaneous administration has been shown to be more effective (Cerletti *et al.* 1980). Subcutaneous administration of low volumes of anaesthetic is also a simpler and subjectively less stressful means of induction than intraperitoneal injection. We therefore examined the effects of sufentanil/medetomidine administered both by the intraperitoneal and subcutaneous routes.

Materials and methods

Twenty-three adult Wistar outbred rats (12 females, 11 males), aged between 6 and 8 weeks, were obtained from the barrier maintained breeding unit of the Comparative Biology Centre of the University of Newcastle upon Tyne. Animals from this colony showed no serological evidence of infection with rat respiratory pathogens. They were housed in a temperature regulated room (20 ± 3°C) with 15 air changes per hour and a 13 h:11 h light–dark cycle (lights on 06:00 h; off 19:00 h). The animals were group-housed (5–6) in solid floored caging (RC1, North Kent Plastics Ltd, Erith, UK; 56 cm × 38 cm, height 18 cm) and received a commercial pelleted diet (R&M No. 3, Special Diets Services Ltd, Essex, UK) and water *ad libitum*. Sawdust ('Gold Chip', BS and S Ltd, Edinburgh, UK) was used as bedding. Body weights were recorded for 2 days prior to anaesthesia.

Anaesthesia

Basic methodology

Medetomidine (Domitor, Pfizer Animal Health, UK) was mixed with water for injection, to allow accurate dosing, and combined with sufentanil (Sufenta Forte, Janssen, Belgium) resulting in a volume for injection of 1.5 or 2.0 ml/kg. Drugs were administered by intraperitoneal or subcutaneous injection into the skin overlying the scapulae on up to four occasions. A minimum of one week elapsed between successive anaesthetics. Following drug administration, the time required for animals to lose the ability to perform a righting reflex when placed in dorsal recumbency was recorded. During anaesthesia, the parameters which were used to assess depth of anaesthesia were the strength of the tail-pinch and pedal withdrawal reflexes. These were assessed by pinching the tip of the tail and the hind-foot metacarpal region between the index finger and thumb. Responses were scored on a scale of 0–3, with complete reflex absence scoring 0, and a strong withdrawal response scoring 3. Complete loss of both the tail-pinch and pedal withdrawal reflexes characterized a plane of anaesthesia sufficient for performing surgical procedures (surgical anaesthesia). The duration of surgical anaesthesia was measured as the latency of return of either the tail-pinch or pedal withdrawal reflexes (a score>0) from the time of loss of the righting reflex. Body temperature was maintained at 36–38°C during anaesthesia using a homeothermic heating blanket (Harvard Apparatus, Edenbridge, Kent, UK). Reflex responses were recorded every 15 min during anaesthesia, as were blood O_2 saturation, heart rate and respiratory rate. Oxygen saturation was measured by pulse oximetry (D-YS clip-on probe; Nellcor, Hayward, California, USA) with the sensor placed on one of the hind-feet. After completion of the main study, six animals were terminally anaesthetized and blood gas tensions were measured in carotid arterial blood samples using a blood gas analyser (Stat Profile 5, Nova Biomedical,

Waltham, Massachusetts, USA). A further six animals from the main study were used to assess the efficacy of the reversal of anaesthesia (see below).

Dose ranging study

In a preliminary study, seven male and seven female rats were given combinations of sufentanil/medetomidine administered subcutaneously or intraperitoneally. Doses were between 10–50 μg/kg for sufentanil and 150–300 μg/kg for medetomidine (Table 1). These data were used to determine an appropriate range of doses for the main study, and these animals were later used in the main study.

Main study

Five dose combinations were selected for more detailed assessment (Table 2). To reduce the effects of variations in responses between animals, and the number of animals used, each rat received three anaesthetics, with an interval of at least one week between successive treatments. The treatment order was randomized for each group of rats, and equal numbers of females and males were used for each dose combination.

Reversal of anaesthesia

Following completion of the main study, six of the rats received 40/150 μg/kg sufentanil/medetomidine subcutaneously. Thirty minutes following induction of anaesthesia, atipamezole (0.5 mg/kg) and butorphanol (0.2 mg/kg) were given as a single subcutaneous injection. Reversal was assessed according to the latency of return of the ability to perform the righting reflex, and by monitoring the respiratory rate.

Table 1 The dose combinations and routes of injection of sufentanil and medetomidine used in the dose ranging study

Sufentanil/ medetomidine (μg/kg)	Route of administration	No. of rats
10/200	Intraperitoneal	2
20/200	Intraperitoneal	2
30/200	Intraperitoneal	2
30/300	Intraperitoneal	2
40/300	Intraperitoneal	1
50/300	Intraperitoneal	1
15/150	Subcutaneous	1
20/150	Subcutaneous	1
40/300	Subcutaneous	1
50/200	Subcutaneous	1

Table 2 The dose combinations of sufentanil and medetomidine which were administered either subcutaneously or intraperitoneally to rats in the main study

Sufentanil/ medetomidine (μg/kg)	Route of administration	Injectable vol. (ml/kg)	No. of rats
40/150	Subcutaneous	1.5	6
50/150	Subcutaneous	1.5	10
50/300	Intraperitoneal	1.5	10
60/300	Intraperitoneal	1.5	6
80/300	Intraperitoneal	2.0	6

Evaluation of blood oxygen saturation

The six rats used in the main study were given a single subcutaneous dose of sufentanil/medetomidine (40/150 μg/kg). Following the onset of surgical anaesthesia, the right carotid artery was cannulated with a 22 G i.v. catheter (Vygon Biovalve, Écouen, France) under local lignocaine analgesia. Between one and five blood samples were obtained from each animal for blood gas analysis. Pulse oximetry data were collected at the time of each sample. The animals were killed with an overdose of pentobarbital on return of the pedal withdrawal reflex.

Data analysis

In addition to calculations of the latency until loss of both the righting reflex and tail-pinch and pedal withdrawal reflexes, mean respiratory rate, blood O_2 saturation and tail-pinch and pedal withdrawal scores were collected at 30 and 60 min post-injection for each dose combination. The mean duration of surgical anaesthesia was also calculated. From previous experience we have found restoration of the righting reflex to be extremely variable, and that a better assessment of the duration of the anaesthesia may be obtained by also recording the latency to first (spontaneous) movement (usually limb movements and partial righting). In the following description of the results obtained from this study, where the number of ani-

mals for which the data were obtained differs from that originally entered into the study (as shown in Table 1), this was due to cases in which the righting reflexes were not abolished. Using each type of assessment, the effects of anaesthesia following intraperitoneal or subcutaneous administration were compared between drug dosages using ANOVA, computed with SPSS software (SPSS, Chicago, USA).

Results

Dose ranging study

The two rats which received 50/200 μg/kg subcutaneously and 50/300 μg/kg intraperitoneally lost the ability to perform the righting reflex and lost their pedal withdrawal responses within 15 min. The animal which received a subcutaneous dose of 50/200 μg/kg became cyanotic and was reversed with atipamezole/butorphanol (0.5/0.2 mg/kg s.c.). The animal which received the 40/300 μg/kg dose subcutaneously was found dead the following day. All other dose combinations failed to result in loss of the pedal withdrawal reflex.

Main study

Time to loss of the righting reflex

Data for the latency of loss of the righting reflex, tail-pinch and pedal withdrawal reflexes are shown in Table 3. All animals given sufentanil/medetomidine subcutaneously at combinations of 40/150 μg/kg or 50/150 μg/kg showed an abolition of the righting reflex within 7 min. Mean times (± SD) for the loss of the righting reflex were not significantly different between subcutaneous doses (3.7 ± 1.2 cf. 3.2 ± 2 min, respectively).

Of 22 rats injected intraperitoneally, five did not lose the ability to perform a righting reflex following placement in dorsal recumbency (two which received 50/150 μg/kg, one which received 60/300 μg/kg and two which received 80/300 μg/kg). Animals which received the 50/300 μg/kg combination lost their righting reflex between 4 and 24 min following injection (11.5 ± 7.8; mean ± SD). Following the administration of 60/300 μg/kg, the time to the loss of righting reflex varied between 1 and 17 min (7.2 ± 6) and between 4 and 15 min (8.2 ± 5.3) in animals given 80/300 μg/kg.

Time to loss of tail-pinch and pedal withdrawal reflexes

All the 16 rats given subcutaneous sufentanil/medetomidine lost their pedal withdrawal and tail-pinch reflexes. For the 40/150 μg/kg combination, the mean times (± SD) for loss of each respective reflex were 11.7 ± 10.6 min and 4.3 ± 2.6 min. For the animals given the 50/150 μg/kg combination, the comparable times were 14.6 ± 6.7 and 7.7 ± 5.3 min. The loss of the pedal withdrawal and tail-pinch reflexes were not significantly different between the subcutaneous doses used ($P > 0.1$).

Of the 22 rats administered drugs intraperitoneally, 12 lost their pedal withdrawal reflex, while 17 lost their tail-pinch reflex. Times to loss of reflexes varied between 3

Table 3 The latency until loss of the righting reflex and tail-pinch and pedal withdrawal reflexes in groups of rats administered combinations of sufentanil and medetomidine either subcutaneously or intraperitoneally. Data are means ± SD

Sufentanil/ medetomidine μg/kg (route)	Latency to loss of pedal withdrawal reflex (min)	Valid *n*	Latency to loss of tail-pinch reflex (min)	Latency to loss of righting reflex (min)	Valid *n*
40/150 (s.c.)	11.7 ± 10.6	6	4.3 ± 2.6	3.7 ± 1.2	6
50/150 (s.c.)	14.6 ± 6.7	10	7.7 ± 5.3	3.2 ± 2	10
50/300 (i.p.)	9.5 ± 3.9	4	11 ± 8	11.5 ± 7.8	8
60/300 (i.p.)	15.3 ± 10.9	4	7.2 ± 6	7.2 ± 6	5
80/300 (i.p.)	13.5 ± 12	4	6.7 ± 3.7	8.2 ± 5.3	4

s.c. = subcutaneous, i.p. = intraperitoneal

and 24 min for the tail-pinch, and 4 and 30 min for the pedal withdrawal reflex. Increasing the dose of sufentanil administered did not result in a significant change in the time required for the loss of either the pedal withdrawal or tail-pinch reflexes.

Respiratory rate and O_2 saturation

Data for mean respiratory rate and blood O_2 saturation levels at 30 and 60 min following drug administration are shown in Table 4. The mean (± SD) resting respiratory rate of the rats used in this study was 79 ± 3 breaths per minute (Hu *et al.* 1992). With the exception of animals given 50/300 μg/kg intraperitoneally, all developed respiratory depression (< 50% of resting rate) within 30 min of drug administration. This persisted for up to one hour in animals which received either 40/150 μg/kg or 50/150 μg/kg subcutaneously, and in the animals which received the 80/300 μg/kg dose combination intraperitoneally. No significant differences were found in respiratory rates at either the 30 or 60 min time samples in comparisons between each subcutaneous dose, or between the three intraperitoneal doses used. Four animals in each of the groups were administered 50/150 μg/kg and 40/150 μg/kg sufentanil/medetomidine subcutaneously, and four animals which received 60/300 μg/kg intraperitoneally were visibly cyanotic. One male which received 40/150 μg/kg subcutaneously showed several periods of apnoea lasting 15 to 30 s, but recovered without the need for reversal with atipamezole/butorphanol. In comparisons between the three intraperitoneal doses used, animals which received the 80/300 μg/kg combination showed a significantly greater reduction in O_2 saturation values at both the 30 and 60 min time points than animals which received the 50/300 μg/kg combination. For the higher dose combination, mean saturation values (± SD) for the 30 and 60 min samples were, respectively, 40 ± 26% and 49 ± 13%, compared to 77 ± 12% and 81 ± 9% in animals which received the 50/300 μg/kg dose combination ($P = 0.015$; $P < 0.001$ for each respective time).

Duration of surgical anaesthesia

All the animals which received subcutaneous sufentanil/medetomidine were surgically anaesthetized. The duration of surgical anaesthesia (mean ± SD) for the 40/150 μg/kg and 50/150 μg/kg combinations administered subcutaneously were 101 ± 49 min and 124 ± 45 min respectively (Table 5). These times were not significantly different between dosages ($P > 0.1$). Equivalent times for the animals which received intraperitoneal sufentanil/medetomidine showed that the highest dose given (80/300 μg/kg) gave a significantly greater duration of surgical anaesthesia (76 ± 23 min) than either the 50/300 μg/kg dose (13 ± 15 min; $P < 0.001$) or the 60/300 μg/kg dose (21 ± 20 min; $P = 0.002$).

Latency of first movement and total sleep time

The times for latency of first movement and total sleep time are also shown in Table 5. Comparisons between the doses given by

Table 4 Measurements of respiratory rate and blood oxygen saturation at 30 and 60 min time intervals following drug administration for each anaesthetic combination used. O_2 saturation values were obtained by pulse oximetry. Data are means ± SD

Sufentanil/ medetomidine μg/kg (route)	Resp. rate at 30 min	Resp. rate at 60 min	O_2 sat at 30 min	O_2 sat at 60 min	Valid *n*
40/150 (s.c.)	29 ± 11	32 ± 12	49 ± 16	52 ± 15	6
50/150 (s.c.)	33 ± 12	39 ± 15	47 ± 16	59 ± 11	10
50/300 (i.p.)	41 ± 15	44 ± 15	77 ± 12	81 ± 9	8
60/300 (i.p.)	35 ± 9	42 ± 12	67 ± 13	75 ± 10	5
80/300 (i.p.)	26 ± 10	27 ± 6	40 ± 26	49 ± 13	4

O_2 sat = oxygen saturation, Resp. = respiratory, s.c. = subcutaneous, i.p. = intraperitoneal

either the subcutaneous or intraperitoneal routes showed no significant differences in the latency of first movement or total sleep time.

Reversal of anaesthesia with atipamezole and butorphanol

All six animals had regained the ability to perform at least one instance of a righting reflex within 7 min of drug administration. Recovery of the righting reflex was abrupt, and animals characteristically adopted a 'straub-tail' response during this period. The mean respiratory rate 15 min before reversal was 32±4 bpm, which rose to 127±15 bpm within 10 min of drug administration. Within 15 min of reversal all animals appeared fully recovered, resuming normal exploratory activities.

Evaluation of reliability of pulse oximeter readings

Table 6 shows data for six rats in which arterial oxygen and carbon dioxide partial pressures (pO_2 and pCO_2) were obtained at the same time as blood oxygen saturation (O_2 sat) measured using pulse oximetry. Inspection of these data indicates that low O_2 sat was generally associated with low arterial pO_2 and elevated pCO_2.

Post-anaesthetic recovery

Animals recovered uneventfully from anaesthesia. No clinically detectable adverse reactions to either subcutaneous or intraperitoneal injection were noted.

Discussion

This study found differences in the characteristics of anaesthesia in animals which receive a range of doses of sufentanil/medetomidine either subcutaneously or intraperitoneally. Subcutaneous administration was more effective than intraperitoneal injection in that all animals given drugs by the former route reached a surgical plane of anaesthesia. There are two plausible explanations for this difference in anaesthetic efficacy. Drugs administered intraperitoneally are subject to a high degree of first-pass hepatic metabolism since they are absorbed into the portal system. A second factor is the possibility of drug loss into the gastrointestinal tract or extraperitoneally. The latter possibility is emphasized by an examination of the number of animals for which latency to first movement and total sleep time data were obtained (Table 5). In several cases, intraperitoneally administered drug dosages were shown to produce surgical anaesthesia in some animals but failed to result in loss of the righting reflex in others. In contrast, subcutaneous administration avoids liver metabolism of the drugs immediately following absorption, allowing the use of a lower dose to achieve an equivalent depth and duration of anaesthesia.

Subcutaneous injections are also much more easily accomplished by inexperienced workers and, based on our subjective assessments of the animals' reactions to injections, this method is seemingly less stressful or painful. Onset of anaesthesia after subcutaneous administration was also more

Table 5 The duration of surgical anaesthesia, latency to first movement and total sleep time in rats for each combination sufentanil/medetomidine administered subcutaneously or intraperitoneally. Data are means ± SD

Sufentanil/ medetomidine µg/kg (route)	Duration of surgical anaesthesia (min)	Latency of first movement (min)	Total sleep time (min)	Valid *n*
40/150 (s.c.)	101±49	141±26	183±42	6
50/150 (s.c.)	124±45	140±31	145±31	10
50/300 (i.p.)	13±15	112±42	130±43	8
60/300 (i.p.)	21±20	119±28	122±29	5
80/300 (i.p.)	76±23	155±12	169±18	4

s.c. = subcutaneous, i.p. = intraperitoneal

rapid than after intraperitoneal administration, indicating that this route is to be preferred.

Although pedal withdrawal responses were quickly abolished after drug treatment, it was apparent that muscle relaxation resulting from the action of medetomidine was not maximized for approximately 10 to 15 min. This delay in peak action is consistent with reports of the pharmacodynamics of medetomidine in the rat (Salonen 1989). It is therefore advisable to incorporate a delay prior to commencing operative procedures when using this anaesthetic regimen. As in other studies (Hu *et al.* 1992) we have demonstrated that administration of μ-agonist opioids such as sufentanil may be associated with an increased risk of respiratory depression. As this is further potentiated by co-administration with medetomidine, this anaesthetic regimen can only be recommended for use in healthy, respiratory disease-free animals. Subsequent clinical experience with this regimen has shown that the hypoxia which occurs can be avoided by provision of oxygen, but pronounced hypercapnia will still be produced.

Pulse oximetry provides a simple and relatively inexpensive means of monitoring respiratory function. However, at low saturations (< 80%) the technique becomes less reliable (Sevinghaus & Naifeh 1987). In practice, this is not usually a significant problem since detection of oxygen saturation levels below 80% requires corrective action. A second factor that can affect pulse oximetry readings is peripheral vasoconstriction, which is known to be produced by medetomidine (Savola 1989). In the present study, pulse oximetry was used to assess the degree of respiratory depression. It was thought advisable to compare pulse oximetry readings with measurements of arterial blood oxygen tension to determine how effective pulse oximetry was in detecting respiratory depression in rats using this particular anaesthetic regimen. Oxygen saturations below 80% were usually associated with arterial pO_2 of < 10 kPa, and those below 40% with pO_2 values of < 6 kPa. It seems likely, therefore, that the low O_2 saturations detected in animals in the main study were associated with severe hypoxia. The direct measurements of arterial blood gases also enabled measurements of pCO_2 in these animals. It can be seen that reduced arterial pO_2 was always associated with hypercapnia. Although pulse oximetry makes no measure of arterial carbon dioxide tensions, when hypercapnia is due to respiratory depression it almost invariably accompanies hypoxia. Table 6 indicates that this trend was present in rats in which direct assessments of arterial blood gases were made. It therefore seems reasonable to assume that the majority of rats in the main study which showed evidence of hypoxia based on pulse oximetry readings were both hypoxic and hypercapnic.

All animals which received drugs subcutaneously recovered uneventfully, and reversal of anaesthesia using atipamezole and butorphanol proved effective, so we consider this anaesthetic regimen to be a safe and effective means of anaesthetizing healthy, respiratory disease-free rats. Furthermore, as

Table 6 Values of simultaneously recorded blood oxygenation saturation, and oxygen and CO_2 partial pressures in each of six rats administered with a single subcutaneous dose of sufentanil and medetomidine (40 μg/150 μg/kg)

Rat	O_2 saturation (%)	pO_2 (KPa)	pCO_2
1	55	6.3	7.7
1	48	5.1	8.0
2	58	7.1	7.8
2	62	6.8	7.7
2	72	10.3	5.8
2	81	10.6	5.8
2	90	10.5	5.8
3	40	6.3	7.4
3	50	7.8	6.4
3	55	7.6	7.2
5	70	11.9	3.5
6	50	9.4	5.7
6	23	5.2	6.6
6	45	6.6	7.4
7	20	5.0	7.9
7	86	11.5	4.5
7	82	10	5.4
7	69	8.7	6.1
7	46	6.4	4.9
7	30	7.0	7.9

butorphanol is a mixed agonist/antagonist, having agonist activity at both sigma and kappa receptors in addition to marked antagonistic properties at mu receptors, a further significant advantage of this anaesthetic technique is the provision of some degree of analgesia following operative procedures (Flecknell *et al.* 1989). If major surgery has been undertaken, additional analgesia can be provided by using NSAIDs such as carprofen or additional doses of butorphanol or other opioids (Flecknell & Waterman-Pearson 2000).

We consider the rapid and complete reversal of anaesthesia to be a significant advantage of the presently investigated anaesthetic technique. During recovery from anaesthesia, small rodents continue to be susceptible to hypothermia, hypoxia and other complications, and require continued monitoring by the anaesthetist. Although administration of atipamezole to partially reverse ketamine/medetomidine or ketamine/xylazine combinations in rats shortens recovery times, the high dose of ketamine needed to produce anaesthesia in this species (typically 75 mg/kg) results in a prolonged (20–30 min) period of profound sedation. Subcutaneous administration was well tolerated by the animals in this study, and no clinically detectable signs of skin irritation were noted. This route of administration offers a simple and convenient means of drug delivery, and in the present study it appeared to produce more consistent effects.

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