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# Observations on the use of medetomidine/ketamine and its reversal with atipamezole for chemical restraint in the mouse

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## Summary

Ketamine and medetomidine produced chemical restraint for minor procedures in mice. Male mice required 50 mg/kg ketamine, 10 mg/kg medetomidine intraperitoneally (i.p.), and females a higher dose of ketamine (75 mg/kg i.p.). The onset of restraint effects, judged by loss of righting reflex, was more rapid in males than females. The effects were reversed using atipamezole (1-2.5 mg/kg). Recovery following administration of atipamezole was more rapid in males than females. We conclude that ketamine/medetomidine, followed by reversal with atipamezole, is an effective technique for chemical restraint in the mouse.

**Keywords** Mouse; ketamine; medetomidine; atipamezole; anaesthesia

The mouse is one of the most frequently anaesthetized animal in laboratory work (Green 1979), often requiring some form of physical or chemical restraint for a variety of experimental protocols, including minor and short-term procedures.

Mice are easily restrained humanely by careful handling (Flecknell 1996) but the use of drugs either by inhalation or parenterally with sedative, tranquillizer, analgesic or hypnotic effect can also be of value.

Among the drugs for parenteral use, ketamine alone (Weisbroth & Fudens 1972, Stunkard & Millar 1974, Green *et al.* 1981) or combined with a number of other compounds such as benzodiazepines (Green 1979, Green *et al.* 1981) and alpha-2 agonists (Green 1979, Green *et al.* 1981, Erhard *et al.* 1984, 1986, Silverman & Ingrafi 1986, Nevalainen *et al.* 1989, Flecknell 1996) has been described as a chemical restraint in different laboratory animal species, allowing a dose-dependent response.

Its additive effects with options have recently been described (Dambisya & Lee 1994).

Medetomidine, 4-1-[(2,3-dimethylphenyl)ethyl]-1H-imidazole is one of the newest alpha-2 adrenergic agonists which closely resembles xylazine in its effects, but is a more selective agonist with a higher affinity for the alpha-2 adrenoceptor (Savola *et al.* 1986). It is licensed in Spain for use in dogs and cats. In combination with ketamine, medetomidine provides muscle relaxation and additional analgesia in cats (Verstegen *et al.* 1989, 1990, Young *et al.* 1990). Doses of 20-40 mg/kg of medetomidine have been recommended as a pre-anaesthetic before administration of intravenous ketamine in laboratory beagles (Raiah *et al.* 1989).

Medetomidine's effects can be reversed by atipamezole, 4-(2-ethyl-2,3-dihydro-1H-inden-2-yl)-1H-imidazole, the most recent and specific alpha-2 antagonist (Scheinin *et al.* 1988). This drug can be given at the end of the procedure to reduce sleep-time. In small mammals such as mice this has the added advantage of reducing

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the risk of hypothermia which is a significant problem in these species (Green 1979).

The initial report on the use of medetomidine-ketamine combination in mice (Voipio *et al.* 1988) indicates that when using this agent, the animals 'failed to reach true surgical conditions'.

## Materials and methods

### Animals

Seventy-eight, locally bred outbred Swiss Webster (SW), 2-3 month old mice of both sexes (35 males, 43 females), weighing between 25-35 g were used. Animals in the main study were anaesthetized as part of an unrelated procedure which required some form of restraint and analgesia of short duration so as to enable plucking of hair and subcutaneous injection. Mice were obtained from the Laboratory Animal Unit of the University of Zaragoza, a non-barrier unit. They were housed in small groups of the same sex, on commercial dust and resin-free shavings (Letica ) at 21 °C, 50% relative humidity, light cycle at 12/12, and at a stocking density of 180 cm<sup>2</sup>/animal. They were fed a commercial pellet diet *ad libitum* (Letica ).

### Ethical approval

The research procedures and housing conditions were in accordance with the Spanish Regulations for the Protection of Experimental Animals (Real Decreto 223/1988).

### Drugs

Medetomidine (Domtor Smith Kline/Beecham, Spain; 1 mg/ml), ketamine (Imalgéne Rhone Merieux; 100 mg/ml) and atipamezole (Antisedan Smith Kline/ Beecham, Spain; 5 mg/ml) were used in this study. Normal physiological saline (Salino Fisiológico Braun ) was used for dilution.

### Apparatus

A veterinary pulse oximeter (Vet Ox Plus SDI, WI, USA) was used for monitoring oxygen saturation (SaO<sub>2</sub>) and heart rate. Rectal temperature was monitored using the rectal probe of an electronic thermometer (Quartz digithermo IMS, UK). One hundred per cent O<sub>2</sub> was delivered to the animal's nose via face mask using

UK) in which the dead space was reduced to a minimum.

### Methods

Mice were taken to the operating theatre in the same cage in which they were housed.

Temperature of the operating theatre was kept at 23°C. The mice were weighed using an electronic balance of 1 g accuracy (postage scale MS-12 Empreco, Barcelona, Spain).

The drugs were diluted with saline in order to ease handling small volumes. The following concentrations were prepared: medetomidine (0.1 mg/ml), ketamine (20 mg/ml), atipamezole (0.25 mg/ml). All three drugs were administered by the intraperitoneal route. Ketamine and medetomidine were given as a mixture in a single injection.

Both the pilot and the main studies were carried out in the morning from 10:00 h to 12:00 h.

### Pilot study

The dose rates required to provide restraint adequate for the project were assessed following randomized injection of one of the following combinations:

- (A) Ketamine 35 mg/kg + medetomidine 0.5 mg/kg
- (B) Ketamine 75 mg/kg + medetomidine 1 mg/kg
- (C) Ketamine 150 mg/kg + medetomidine 1 mg/kg

The combinations were given to a pilot group of 6 mice (3 males and 3 females). A total of 18 trials were performed. Each animal received the three doses with a five-day interval between trials. Time of loss of righting reflex and response to toe-pinprick was assessed by an independent observer with experience in work with laboratory mice.

Oxygen saturation was not monitored in all animals because of technical problems with the probe. When successful measurements were obtained, figures as low as 80% were observed if

they were breathing room air, so the use of oxygen was instituted during the pilot as well as during the main study as soon as the animals lost the righting reflex. There was no obvious cyanosis of visible mucous membranes or of the surface of the paw when breathing room air.

### Main study

A dose of ketamine 40 mg/kg/medetomidine 1 mg/kg for males and ketamine 75 mg/kg/medetomidine 1 mg/kg for females was used to inject 72 mice in the main study.

### Assessing the effects

T0 was the time at which first injection was given, T1 the time at which the righting reflex was lost, and T2 when atipamezole was given. This was calculated by adding 5 min (procedure duration) to T1 in males and females. T3 was the time when the righting reflex reappeared and T4 when total recovery was produced.

The following observations were made and recorded from the pilot group as well as from the rest of the animals by the same independent observer:

- (1) Rectal temperature ( $^{\circ}\text{C}$ ) at T0 and T2
- (2) T1 and T3
- (3) A simple score system was established to grade the presence or not of opisthotonus, tail rigidity, uncoordination and muscle relaxation. This system scored from 0 (total absence) to 4 (fully present and clearly visible) of each one of these effects.
- (4) T4 time of total recovery at which the animal escaped when touched.

Student's unpaired test (Statview) was used for all statistical analyses. All results are given as  $\pm$  SD.

## Results

### Pilot study

Dose A was insufficient for females. Dose C caused a very deep plane of anaesthesia in both males and females, with a dangerously prolonged recovery period unnecessary for our needs. Dose B was found to be more appropriate for both males and females, but it was found possible to reduce the ketamine dose to 40 mg/kg which

proved to be sufficient for males, although in females 75 mg/kg was still needed.

Atipamezole was administered to reverse medetomidine's effects at a starting dose of 1 mg/kg and then, if needed, at an incremental dose of 0.25 mg/kg.

### Main study

Mean rectal temperature at T0 was  $36.6^{\circ}\text{C} \pm 0.2$  for males and  $36.3^{\circ}\text{C} \pm 0.2$  for females and  $32.3^{\circ}\text{C} \pm 1.4$  for males and  $31.7^{\circ}\text{C} \pm 0.9$  for females at T2. Table 1 shows mean T1, T3 and T4 for males and females. Doses of atipamezole which produced a satisfactory reversal, varied from 1-1.75 mg/kg in males and 2.25-2.50 mg/kg in females. Tail rigidity was scored at 2.5 in both sexes 1 min after the injection of atipamezole. Muscle relaxation and uncoordination were scored at 3.5 for males and females just before the antagonist was administered. No opisthotonus were observed.

Pulse oximeter measures proved unreliable, and insufficient data were obtained for inclusion in the analysis.

Males lost their righting reflex (T1) significantly more rapidly ( $P < 0.0001$ ) than females. Consequently the time of administration of atipamezole was sooner. Time for reappearance of the righting reflex after atipamezole administration (T3) was significantly ( $P < 0.005$ ) more rapid in males than females as was time to total recovery ( $P < 0.001$ ).

## Discussion

Mouse restraint and anaesthesia have to deal with some major changes affecting the normal

**Table 1 Mean  $\pm$  SD times (minutes) observed for male (n=35) and female (n=43) mice**

Time	Male (N=35)	Female (n=43)
T1	$1.5 \pm 0.56$	$2.8 \pm 1.5$
T3	$7.43 \pm 12.81$	$12.33 \pm 17.18$
T4	$23 \pm 6.11$	$32.26 \pm 14.35$

T1 =time at which the righting reflex was lost  
 T3=time at which the righting reflex reappeared  
 T4=time at which total recovery was registered

physiology of the animal. Hypothermia and hypoxia have been claimed to be the main cause of anaesthetic death in mice (Green 1979), although limited information is available in the literature on mouse anaesthesia in comparison with domestic species.

Although the use of inhalational techniques have been described as the 'most simple and effective means of producing a short period of anaesthesia' (Waynforth & Flecknell 1992), this requires appropriate equipment and may be impractical when dealing with a large number of animals simultaneously. There is also the risk of polluting

the operating theatre environment with the anaesthetic gases, although this should not be a problem if the right equipment is used.

In the present study a short period (5-10 min) of restraint and analgesia was needed, so the technique of reversible anaesthesia was the most suitable method that could be found. This makes the work easier when a large group of animals have to be handled simultaneously (Green 1979), allows a more rapid recovery and prevents the development of long-lasting hypothermia (Flecknell 1996, Waynforth & Flecknell 1992).

The pharmacological development of more potent alpha-2 agonists such as medetomidine (Savola *et al.* 1986), offers new possibilities to the use of these agents alone or in combination with some of the classical and widely used drugs such as ketamine, especially for short-term procedures (up to 20 min). Moreover the use of the more specific alpha-2 antagonist atipamezole allows for the rapid recovery of the animals reducing the possibilities of deleterious side-effects.

Ketamine-medetomidine combination proved to be very useful as a method of chemical restraint in our research protocol. This is in accordance with other authors' observations (Voipio *et al.* 1988), who reported that this combination produced light anaesthesia and good immobilization, although the effects of atipamezole were not evaluated in their study.

Increased diuresis is one of the well recognized side effects of xylazine and medetomidine in rats (Waynforth & Flecknell 1992) but we did not observe this in any of the animals, nor did we observe increased salivary

secretion.

The most marked undesirable effect was hypothermia (4-4.6 °C reduction) which is also seen when other anaesthetic drugs are used in the mouse.

A sex-related difference was observed in the time of onset of loss of the righting reflex. Other authors have measured the duration of sleep time (duration of loss of righting reflex) between sexes and strains, but we could find no published data measuring the loss time. Male mice of several strains had longer sleep time than females after pentobarbitone (Brown 1959, Lovell 1986) or hexobarbitone (Rümke & Noordhoek 1969). Females had a tendency to sleep longer after intraperitoneal medetomidine-ketamine combination (Voipio *et al.* 1988). In the present study, females of the SW strain took almost twice as long as males to lose the righting reflex, and to recover it after atipamezole injection. They needed 55% more ketamine and 70% more atipamezole than males. Males needed an extra 23 min for the escape reflex to reappear and females recovered it in another 32 min. Tail rigidity, probably due to some residual ketamine, was the only effect observed once the animals had recovered consciousness.

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