



Evaluation of anaesthetic stress following administration of dexmedetomidine and its combinations with midazolam, fentanyl and ketamine in dogs

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ABSTRACT

The study was conducted on 16 apparently healthy adult dogs of either sex, randomly divided into 4 groups, each containing 4 animals. Animals of group A received dexmedetomidine (20 µg/kg) alone. Midazolam (0.2 mg/kg), fentanyl (4 µg/kg) + midazolam (0.2 mg/kg) and ketamine (10 mg/kg) + fentanyl (4 µg/kg) + midazolam (0.2 mg/kg) were administered along with dexmedetomidine (20 µg/kg) in the animals of groups B, C and D, respectively. All the drugs were given simultaneously by intramuscular route. Differential leucocytic count, plasma glucose, insulin and cortisol were measured at baseline (0 min) and at 30, 60 and 120 min after the drug administration. Neutrophilia was observed only for a very short period. Plasma insulin levels significantly decreased whereas a significant increase in the plasma glucose towards the end of the study period was observed except in group C in which it decreased. Cortisol levels were nonsignificantly lower than the base values throughout the study in all the groups. It could be concluded that dexmedetomidine and its combinations with midazolam, fentanyl and ketamine do not threaten homeostasis and can obtund the stress response associated with handling and anaesthetic procedures in dogs.

Key words: Dexmedetomidine, Dogs, Fentanyl, Midazolam, Stress indicators

Stress is a state of threatened homeostasis induced by various physical or psychological factors including trauma, anaesthesia, surgery and infection. It is expressed in the form of altered haemato-biochemical profile such as neutrophilia, increase in catecholamines, cortisol, glucose (Tabuchi *et al.* 1989). The increase in the stress hormones is considered undesirable because they promote haemodynamic instability and intraoperative and postoperative catabolism (Bovill *et al.* 1984). Most of the sedative and analgesic drugs used in veterinary clinical practice are not able to reduce the level of stress response in the animals (Vaisenen *et al.* 2002). Alpha-2 agonists like xylazine and medetomidine delay ACTH and cortisol response to stress (Frank *et al.* 1992, Benson *et al.* 2000). However there seems to be scarcity of the similar information about dexmedetomidine. Midazolam, fentanyl and ketamine are frequently used with alpha-2 agonists to increase the sedation and analgesia in several studies. Fentanyl suppresses metabolic and endocrine responses to stress but ketamine stimulates pituitary adreno-cortical axis (Oyama *et al.* 1970).

The purpose of this study was to assess the level of anaesthetic stress response as evidenced by changes in neutrophil count, cortisol, glucose and insulin brought about by dexmedetomidine and its combinations with midazolam, fentanyl and ketamine in dogs.

MATERIALS AND METHODS

Client owned, apparently healthy mixed breed adult dogs (16) of either sex, were used in the study. The animals were divided randomly into 4 groups each group containing 4 animals designated as A, B, C and D. Dexmedetomidine (20 µg/kg body wt) alone was administered in group A, a combination of dexmedetomidine (20 µg/kg body wt) and midazolam (0.2 mg/kg body wt) was administered in group B, combination of dexmedetomidine (20 µg/kg body wt), midazolam (0.2 mg/kg body wt) and fentanyl (0.004 mg/kg body wt) was used in the animals of group C, and a combination of dexmedetomidine (20 µg/kg body wt), midazolam (0.2 mg/kg body wt), fentanyl (0.004 mg/kg body wt) and ketamine (10 mg/kg body wt) were used in the animals of group D. All the drugs were given simultaneously by intramuscular route using separate syringes. After administration of drug (s) the animal was left in a large room to allow onset of effects without disturbance and time of induction was recorded.

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Blood (2 ml) was collected from the cephalic vein of each animal in disposable plastic syringes, containing heparin (1: 1000) at 0 min (baseline), 30, 60 and 120 min after the administration of the drugs. Blood samples for base values were taken in the presence and control of animal owner to minimize struggling. A drop of blood was used for preparation of blood film for estimation of differential leucocytic count to record neutrophil count (%) as described by Schalm *et al.* (1975). The remaining blood was centrifuged at 3000 rpm for 5 min and plasma was separated and stored at - 20 °C until assayed. This plasma sample was subjected to the estimation of plasma insulin (μ IU/ml) and cortisol (nmol/L) by RIA procedure using commercially available RIA kit. Another blood sample (1 ml) was collected from each animal at the same intervals in clean dry vials containing sodium fluoride. The plasma from the blood was separated by centrifugation and was stored at -20 °C until used for estimation of plasma glucose (mmol/L) by GOD/POD (Trinder 1969) using commercial kits.

SPSS software version 15.0 was used for data analysis. One way analysis of variance and Duncan's multiple range test (DMRT) were used to compare the means at different time intervals among different groups. Paired "t" test was used to compare the mean values at different intervals with their base values in each group. In each analysis, the differences were considered significant at a value of $P < 0.05$.

RESULTS AND DISCUSSION

After intramuscular injection of the drugs the animals of different groups showed signs of action of the drugs in 4.5 ± 0.96 min in group A, 3.25 ± 0.63 min in group B, group C 2.5 ± 0.29 min and 1.50 ± 0.29 min in group D. Significantly lower time of onset of effects was recorded in groups C and D as compared to groups A and B.

Neutrophil count: Neutrophils increased significantly ($P < 0.05$) initially in groups B, C and D however, these values were only nonsignificantly higher than the respective baseline values during most of the observation period. Neutrophil count in group A fluctuated near the baseline with changes which were statistically nonsignificant. The differences among the groups were also nonsignificant. Any increase in neutrophils was associated with a corresponding decrease in the lymphocyte count. Values of neutrophils and lymphocytes, however, always remained within physiological limits (Table 1).

The initial increase in neutrophils and a corresponding decrease in lymphocytes may be associated with initial excitement due to handling of the animals and slight pain at the injection site. Handling and confinement in unfamiliar surroundings may cause release of epinephrine leading to the release of neutrophils from bone marrow (Rosin 1981). Some of the anaesthetics themselves are also known to induce release of epinephrine and cortisol which may cause neutrophilia (Fauci *et al.* 1976). Since significant neutrophilia

was not observed during the study it could be inferred that the combination did not cause marked epinephrine release to affect the release of neutrophils in the circulation.

Insulin: Plasma insulin levels were significantly reduced in all the groups as compared to the respective base values, however, no significant differences were recorded among the groups. In group C, although a substantial decrease in the insulin occurred as in other groups but it was significant at 120 min only (Table 2).

Alpha-2 agonists stimulate alpha-2 receptors on pancreatic beta cells, which lead to inhibition of insulin release (Burton *et al.* 1997). In earlier studies also, serum insulin levels decreased significantly for several hours following medetomidine administration in dogs and cats (Ambrisko and Hikasa 2002, Kanda and Hikasa 2008). The significant reduction in plasma insulin thus may be attributed mainly to the effects of dexmedetomidine on alpha-2 adrenoreceptors. No significant differences between the groups also suggested that the other drugs either had no effect on plasma insulin or had only very little effect, which might have been masked by the action of dexmedetomidine.

Glucose: In the animals of group A, plasma glucose concentration remained unchanged up to 30 min, followed by a slight decrease at 60 min but a significant increase was recorded at 120 min of the study (Table 3). Alpha-2 agonists

Table 1. Mean $\pm$ SE values of neutrophil count (%) in different groups at different intervals

Group	Time (min)			
	0	30	60	120
A	69.00 $\pm$ 3.00	70.75 $\pm$ 2.43	67.50 $\pm$ 1.44	68.50 $\pm$ 0.65
B	65.25 $\pm$ 2.29	71.00* $\pm$ 1.08	69.13* $\pm$ 1.42	69.75 $\pm$ 1.27
C	68.88 $\pm$ 2.44	72.25* $\pm$ 2.06	67.13 $\pm$ 1.90	67.88 $\pm$ 0.85
D	64.88 $\pm$ 3.32	73.25* $\pm$ 2.06	68.00 $\pm$ 2.20	69.75 $\pm$ 1.53

Values with different alphabets differ significantly ($P < 0.05$) at corresponding intervals; *, differ significantly ($P < 0.05$) from respective base values; **, differ significantly ($P < 0.01$) from respective base values.

Table 2. Mean $\pm$ SE values of plasma insulin (μ IU/mL) in different groups at different intervals

Group	Time (min)			
	0	30	60	120
A	17.08 $\pm$ 1.65	5.93** $\pm$ 0.46	4.30** $\pm$ 0.45	4.80** $\pm$ 0.28
B	18.39 $\pm$ 2.75	5.25* $\pm$ 0.59	5.65* $\pm$ 0.46	6.27* $\pm$ 0.67
C	15.75 $\pm$ 4.12	5.74 $\pm$ 1.84	4.71 $\pm$ 1.30	4.29* $\pm$ 1.05
D	15.06 $\pm$ 2.14	5.60* $\pm$ 0.57	4.60** $\pm$ 0.49	4.54* $\pm$ 0.29

Values with different alphabets differ significantly ($P < 0.05$) at corresponding intervals; *, differ significantly ($P < 0.05$) from respective base values; **, differ significantly ($P < 0.01$) from respective base values.

can cause an increase in plasma glucose concentration due to inhibition of insulin secretion by their action on alpha-2 receptors in beta cells of pancreas (Burton *et al.* 1997). Raekallio *et al.* (2005) reported a decrease in blood glucose concentration at 30 min after dexmedetomidine administration, which returned to baseline at 90 min in beagle dogs. They did not observe increase in blood glucose in their study possibly as the period of observation was not long enough to permit detection of peak glucose concentrations. Peak glucose levels were detected at 2 h after administration of medetomidine (Ambrisko and Hikasa 2002) as was the case in the present study. In group B animals, plasma glucose concentration decreased at 30 min, followed by nonsignificant increase at 60 min and a significant increase at 120 min. In a study in dogs midazolam caused a nonsignificant increase in blood glucose level throughout the study period (Butola and Singh 2007). In group B of the present study, plasma glucose followed the same pattern as that of plasma glucose of group A and thus it was conjectured that these effects were primarily attributed to the action of dexmedetomidine. Plasma glucose decreased in the animals of group C during the period of observation. In dogs receiving preoperative transdermal fentanyl patches, blood glucose concentration was reported to be significantly lower than in those not receiving the patch (Igna *et al.* 2003). In another study in diabetic dogs lacking insulin, μ receptor activation by tramadol increased the utilization of glucose and/or decrease hepatic gluconeogenesis, so as to lower plasma glucose (Chang *et al.* 2001). Thus the administration of fentanyl in the animals of group C could have resulted in the lower values of plasma glucose. A significant increase in plasma glucose in the animals of group D was recorded towards the end of the observation period. This increase was possibly attributed to the predominant action of ketamine. Ketamine increased glucose concentration in plasma (Ylitalo *et al.* 1976). A decrease in plasma insulin caused by the dexmedetomidine might have also contributed to the increase in plasma glucose towards the end of the study in different groups.

Cortisol: Plasma cortisol values decreased in all the groups

Table 3. Mean $\pm$ SE values of plasma glucose (mmol/L) in different groups at different intervals

Group	Time (min)			
	0	30	60	120
A	4.03 ^a $\pm$ 0.15	4.07 $\pm$ 0.09	3.90 $\pm$ 0.68	5.75 ^a $\pm$ 0.27
B	3.87 ^a $\pm$ 0.30	3.70 $\pm$ 0.42	4.20 $\pm$ 0.43	5.34 ^a $\pm$ 0.44
C	5.55 ^b $\pm$ 0.70	4.42 $\pm$ 0.16	3.75 $\pm$ 0.13	3.89 ^c $\pm$ 0.15
D	3.50 ^a $\pm$ 0.28	3.51 $\pm$ 0.32	4.50 $\pm$ 0.12	4.65 ^a $\pm$ 0.06

Values with different alphabets differ significantly ($P < 0.05$) at corresponding intervals; *differ significantly ($P < 0.05$) from respective base values; **differ significantly ($P < 0.01$) from respective base values.

Table 4. Mean $\pm$ SE values of plasma cortisol (nmol/L) in different groups at different intervals

Group	Time (min)			
	0	30	60	120
A	127.70 ^a $\pm$ 40.22	84.48 $\pm$ 41.59	54.99 $\pm$ 22.92	45.93 $\pm$ 31.20
B	40.70 ^b $\pm$ 6.35	33.84 $\pm$ 12.10	26.94 $\pm$ 12.61	57.42 $\pm$ 14.09
C	56.34 ^b $\pm$ 10.60	69.09 $\pm$ 29.97	72.69 $\pm$ 29.56	46.64 $\pm$ 20.11
D	19.11 ^b $\pm$ 3.38	30.02 $\pm$ 4.20	18.43 $\pm$ 3.12	10.36 [*] $\pm$ 1.77

Values with different alphabets differ significantly ($P < 0.05$) at corresponding intervals; *differ significantly ($P < 0.05$) from respective base values; **differ significantly ($P < 0.01$) from respective base values.

from the baseline values but this decrease was nonsignificant in all groups except in group D, in which, cortisol level decreased significantly at 120 min of observation (Table 4).

Cortisol is measured as an indicator of stress response. Under stressful conditions cortisol level increased (Rosin 1981). Alpha-2 agonists directly or indirectly obtund the stress response when administered systemically. Reduction in the perception of stressors is achieved indirectly through sedation and analgesia while directly by inhibiting neuroendocrine response. ACTH and cortisol response to ovariectomy was delayed in dogs that received medetomidine preoperatively (Benson *et al.* 2000). Similarly, administration of dexmedetomidine resulted in a decrease in plasma cortisol concentration in dogs that received dexamethasone with or without exercise (Raekallio *et al.* 2005). Fentanyl is a known suppressor of endocrine and metabolic responses to surgical stress (Bovill *et al.* 1984), while on the other hand ketamine administration results in stimulation of pituitary-adrenocortical axis producing an increase in the plasma cortisol level (Oyama *et al.* 1970). The reduced levels of cortisol thus may be attributed mainly to the protective actions of dexmedetomidine and fentanyl which even prevented ketamine induced increase in plasma cortisol concentration.

Our results indicated that administration of dexmedetomidine and its combinations with midazolam, fentanyl and ketamine do not threaten the homeostatic balance or do not stimulate hypophysis-pituitary adrenocortical axis and thus minimise the stress response associated with handling and anaesthetic procedures. Dexmedetomidine and combinations therefore may be employed safely for sedation, analgesia and anaesthesia in dogs.

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