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Influences of type of anaesthesia on cortisol, β-endorphin and heart rate in pigs

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Summary — Two experiments were carried out using a total of 20 growing pigs of approximately 20 kg in order to investigate the effect of different types of anaesthesia (metomidate versus ketamine) on plasma levels of cortisol and β-endorphin on the heart rate. The animals were housed individually within a zone of thermal neutrality. Feed and water were available ad libitum. After becoming accustomed to human contact (being approached and touched), a catheter was implanted in the jugular vein, exteriorized in the neck region. This operation was performed under halothane anaesthesia. After recovery, hourly blood samples were taken over a 27 hour period, and cortisol and beta-endorphin concentrations were measured in the sample plasma. After this reference period, half of the animals were anaesthetized with metomidate, the other half with ketamine, and blood samples were taken at regular intervals. During a second experiment the heart rate was monitored after being anaesthetized as described before. An influence of the type of anaesthesia was observed on the plasma concentration of cortisol, but no effect was observed with respect to the β-endorphin concentration. A fivefold increase of cortisol concentration was observed after injection with ketamine, while a twofold increase was observed after injection with metomidate. This finding suggests a suppressive effect of metomidate on the plasma cortisol levels. A fivefold increase was also observed for the β-endorphin concentration after injection with either ketamine or metomidate. The increase in the heart rate was related to the awakening activity, which was more difficult after the ketamine injection.

pig / anaesthesia / cortisol / β-endorphin / heart rate

Résumé — Influence du type d'anesthésie sur le taux de cortisol, de β-endorphines et sur le rythme cardiaque chez le porc. *Au cours de deux expérimentations l'effet de l'anesthésie (métomidate ou kétamine) a été recherché sur les taux plasmatiques de cortisol et de β-endorphine, ainsi que sur le rythme cardiaque. Vingt porcelets d'environ 20 kg de poids vif ont été placés dans des loges individuelles. Les conditions thermiques étaient optimales ; la nourriture et l'eau étaient distribuées ad libitum. Quand les porcelets ont été habitués au contact humain, un cathéter a été implanté dans la veine jugulaire sous anesthésie à l'halothane. Après la guérison, chaque heure, un échantillon de sang a été prélevé pendant une période de 27 heures pour déterminer les valeurs de référence. Puis la moitié des porcelets a été anesthésiée par une injection de métomidate ou de kétamine, suivie par la collecte des échantillons de sang. Dans le plasma les taux de cortisol et de β-endorphine ont été déterminés. Au cours d'une autre épreuve, le rythme cardiaque a été mesuré avant, pendant et après l'anesthésie. Le type d'anesthésie a eu une influence sur le taux de cortisol et le rythme cardiaque. Le taux de cortisol a augmenté cinq fois après une injection de kétamine, de même que le taux de β-endorphine après une injection de kétamine ou métomidate. Le taux de cortisol a augmenté seulement deux fois après une injection de métomidate. Ce résultat suggère un effet suppressif du métamidate sur le taux de cortisol plasmatique. L'augmentation du rythme cardiaque était liée à l'activité d'éveil, plus laborieuse en cas d'injection de kétamine.*

porc / anesthésie / cortisol / β-endorphine / rythme cardiaque

INTRODUCTION

The release of ACTH and β-endorphin from the anterior pituitary gland during stress reactions is controlled by the corticotrophin releasing hormone (CRH) (Bagdy et al, 1990a; Vale et al, 1981). Adrenocorticotropic hormone (ACTH) induces the production and release of glucocorticoids such as cortisol from the adrenal cortex. Plasma β-endorphin also originates from the intermediary lobe of the pituitary gland (Bagdy et al, 1990b; Murburg et al, 1993). The type of stress may determine however, the level of production of β-endorphin from the anterior or intermediary lobe of the pituitary gland (Kjaer et al, 1995). Diurnal rhythmicity has been reported for plasma levels of β-endorphin in rats (Bagdy et al, 1991) and humans (Iranmesh et al, 1989; Veldhuis et al, 1990), and for plasma cortisol levels in pigs (Becker et al, 1985; Bottoms et al, 1972; Dalin et al, 1993; Favre and Moatti, 1977). In humans, the stimulation of the hypothalamus-pituitary-adrenal axis by ketamine and other anaesthetics is well known (Monk et al, 1992; Oyama et al, 1970; Preziosi and Vacca, 1988). An increase in plasma cortisol levels normally starts about 10 min after injection. At the level of the adrenal cortex, however, etomidate blocks the stimulating effect of ACTH, thus inhibiting the increase in cortisol levels (Preziosi and Vacca, 1988).

Several drugs are used for pig anaesthesia. Azaperone, a butyrophenone derivative, acts as a neuroleptic drug. It is particularly useful in pigs as a premedicant for anaesthesia or for reducing distress (Green, 1979). Metomidate and etomidate are both hypnotic agents. They have marked muscle relaxant properties in pigs, but produce no analgesia. In combination with azaperone however, they produce a state that resembles general anaesthesia (Callear and Van Gestel, 1973; Green, 1979). Etomidate is not often used in pigs, mainly because it has to be injected intravenously (Holzchuh and Cremonesi, 1991). Ketamine hydrochloride produces loss of consciousness and peripheric analgesia, but no muscle relaxation. Therefore, azaperone is used in combination with ketamine to complete analgesia and to reduce muscle tone (Green, 1979). It is therefore necessary to consider one's research objectives in order to select an appropriate type of anaesthesia.

The aim of our research was to find out whether metomidate has the same suppressing effect as etomidate on plasma cortisol levels and rhythmicity. Ketamine was used as a reference method because it increases cortisol plasma level. Plasma β-endorphin, the release of which is concomitant with ACTH (Vale et al, 1981), and which is less affected by storage conditions after blood sampling than ACTH (ICN Biochemicals, 1984), was evaluated as a second parameter. Heart rate, which is affected by anaesthetic agents, was also measured.

MATERIALS AND METHODS

Experiment 1

Eight Large White x Landrace piglets (four male, four female) weighing between 18 and 20 kg were housed individually on straw. Food and water were provided ad libitum. Lights were on continuously in order to allow blood sampling. After becoming accustomed to human approach and contact, a catheter was inserted in the jugular vein and exteriorized in the neck region. The operation was performed under halothane anaesthesia. Patency of the catheters was maintained by daily flushing with 50 units/mL heparinized saline. When the pigs had recovered, three days after the operation (Dalin et al, 1993), hourly blood sampling started at noon and continued for 27 h. The first three samples allowed the animals to become used to frequent sampling and were not included in the results. About 10 mL of blood was collected in cooled EDTA tubes and immediately centrifuged. Plasma was stored at –20 °C until further analysis. Two days later, the animals were randomly divided into two groups (four pigs each). One group was injected intravenously with azaperone (Stresnil, 40 mg/mL, Janssen Pharmaceutica, Beerse, Belgium) in combination with ketamine (Vetalar, 100 mg/mL, Parke Davis, Gwent, UK), and the other group with azaperon in combination with metomidate (Hypnodyl, 50 mg/mL, Janssen Pharmaceutica, Beerse, Belgium). The experimental protocol and doses are shown in table I. On the first experimental day the anaesthetic was injected intravenously at 9 am and 3 pm. Blood samples were taken by the i/v catheter at 60 min and 1 min prior to the administration of anaesthetic and 5, 30, 60 and 120 or 180 min after its administration. Samples were handled as previously described. The same procedure was repeated two days later with an intramuscular injection of azaperone and ketamine and an intraperitoneal injection of metomidate (table I).

Plasma hormone levels were determined with commercially available kits. Because the assays needed to be carried out two months after sample collection, β-endorphin was analysed instead of ACTH. β-Endorphin is released concomitantly with ACTH, but is less affected by duration of storage of the sample at –20 °C when no preservatives are added (ICN-Biochemicals, 1984). Plasma cortisol was evaluated by enzyme immunoassay (Biogenesis Ltd, Bournemouth, UK). This heterogeneous enzyme immunoassay method is based on the principle of competitive binding. Cortisol is released from serum binding proteins in a two-step process. First, the dilution of the serum protein in the assay weakens its binding capacity. Secondly, the higher affinity antibody competes more effectively for the cortisol than the weaker binding serum proteins. The total amount of cortisol present is free to compete with an enzyme-labelled cortisol for a limited number of available binding sites on an antibody specific for cortisol. The bound and free enzyme-labelled cortisol fractions are separated. The bound enzyme is quantified by the enzymatic conversion of a specific substrate to a coloured product. The colour intensity is inversely related to the amount of cortisol in the sample. Intra- and inter-assay variability were respectively 4.9 ± 1.2% and 6.4 ± 1.7%. The mean recovery value was 99 ± 2%, and the cross-reactivity was 100% for cortisol, 14.8% for 11-deoxycortisol, 11.5% for corticosterone and lower than 2% for other related compounds. Total plasma β-endorphin content was evaluated by Radio immuno assay (RIA) (Peninsula Laboratories, St Helens, UK). This kit is designed to measure specifically β-endorphin (rat) levels, and its related peptides by a competitive RIA. Intra- and inter-assay variability were respectively 7.6 ± 3.3% and 9.4 ± 4.1%. The mean recovery value was 88 ± 4%. Cross-reactivity for porcine β-endorphin was 100%. Figure 1 shows the similarity of the dilution curves for three samples of pig plasma.

Experiment 2

Twelve female pigs (≈ 20 kg) of an homozygous halothane negative line were used (Seghers Hybrid, Buggenhout, Belgium). Six were anaesthetized with an intramuscular injection of azaperone (2 mg/kg) and an intraperitoneal injection of metomidate (10 mg/kg). The others were injected with azaperone and ketamine (10 mg/kg IM) (Imalgene, Rhône Mérieux, France). After anaesthesia, an ambulatory heart rate monitoring device, using five electrodes positioned on the chest, was fixed on the back of the pig (Villé et al, 1993). Data acquisition covered the whole measuring period. The pigs were housed individually within their zone of thermal neutrality and were fed ad libitum.

Statistics

The individual pigs were chosen at random from different litters within the population of pigs available. Because the pigs themselves were the experimental units and randomly allocated to the fixed main effects, statistical analysis was based on a randomized lay-out. Treatment effects on mean values were contrasted by using analysis of variance within a generalized linear model, including the time factor as repeated measures were made in the same animal (SAS Guide, 1988). Statistical differences between the times of day were expressed as two-tailed probability values within a Students *t*-test procedure.

RESULTS

Influence of type of anaesthesia on plasma cortisol and β-endorphin (Experiment 1)

The two days prior to the anaesthetics injection were considered as reference days. Plasma cortisol levels were high (up to

Table I. Experimental protocol of experiment 1.

	Day 1		*Day 3*	
Time	*Group 1*	*Group 2*	*Group 1*	*Group 2*
08.00	S1	S1	S1	S1
08.59	S2	S2	S2	S2
09.00	Az IV 1 mg Ke IV 2 mg	Az IV 1 mg Me IV 2.5 mg	Az IM 2 mg Ke IM 10 mg	Az IM 2 mg Me IP 10 mg
09.05	S3	S3	S3	S3
09.30	S4	S4	S4	S4
10.00	S5	S5	S5	S5
12.00	S6	S6	S6	S6
14.00	S7	S7	S7	S7
14.59	S8	S8	S8	S8
15.00	Az IV 1 mg Ke IV 2 mg	Az IV 1 mg Me IV 2.5 mg	Az IM 2 mg Ke IM 10 mg	Az IM 2 mg Me IP 10 mg
15.05	S9	S9	S9	S9
15.30	S10	S10	S10	S10
16.00	S11	S11	S11	S11
17.00	S12	S12	S12	S12

S = sample, Az = azaperone, Ke = ketamine, Me = metomidate, IV = intravenous, IM = intramuscular, IP = intraperitoneal, dose = mg/kg body weight.

80 ng/mL) from 00.00–12.00 h, and low (down to 30 ng/mL) between 13.00–24.00 h ($P < 0.001$) (fig 2). The measurements of β-endorphin were too close to the detection limits of the radioimmunoassay (< 10 pg/mL), and could not be used for statistical analysis. A fivefold increase in cortisol levels was observed within 30 min after the ketamine injection in the morning, and a threefold rise in the afternoon ($P < 0.005$) (fig 3). About 2 h after the injection, the plasma values returned to normal levels. Injection with metomidate produced only a twofold increase in basal levels ($P < 0.05$), but the return to basal level took about 6 h (fig 3). A statistically significant ($P < 0.005$) fivefold increase in plasma β-endorphin 60 min after injection is shown in figure 4. About 2 h after the injections, the concentrations returned to basal levels. This return took a little bit longer after metomidate anaesthesia. The method of drug injection (IV or IM/IP) had no significant effect.

Influence of type of anaesthesia on heart rate (Experiment 2)

Figure 5 presents the hourly mean heart rate (beats/min) as a function of time after injection with metomidate or ketamine. Heart rate is strongly related to body activity during awakening. As early as 30 min after the ketamine injection the pigs demonstrated

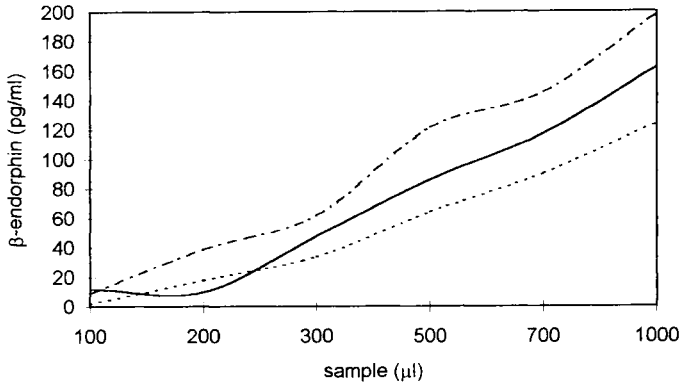

Fig 1. Dilution curves of three pig plasma samples. —— sample 1; ····· sample 2; – · – · sample 3.

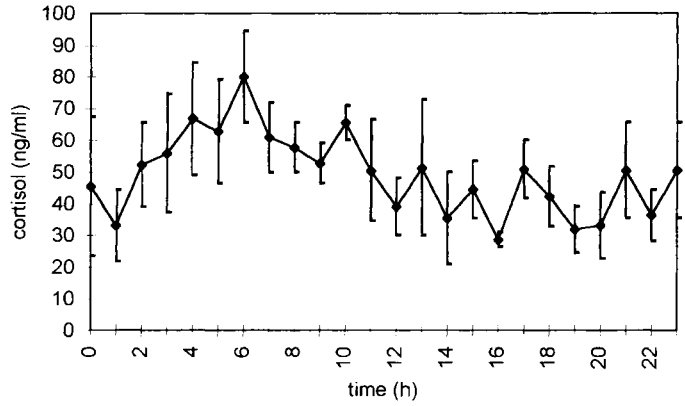

Fig 2. Circadian rhythm of plasma cortisol values (mean values with standard error of the mean) during two reference days (eight pigs).

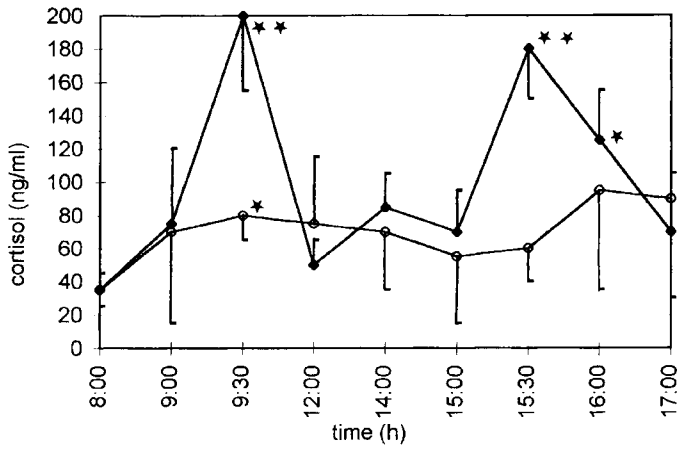

Fig 3. Plasma cortisol levels (mean values with standard error of the mean for four pigs per treatment) after injection with metomidate or ketamine (at 09.00 and 15.00 h) (* = $P < 0.05$; ** = $P < 0.005$, with respect to the baseline). —o— metomidate; —◆— ketamine.

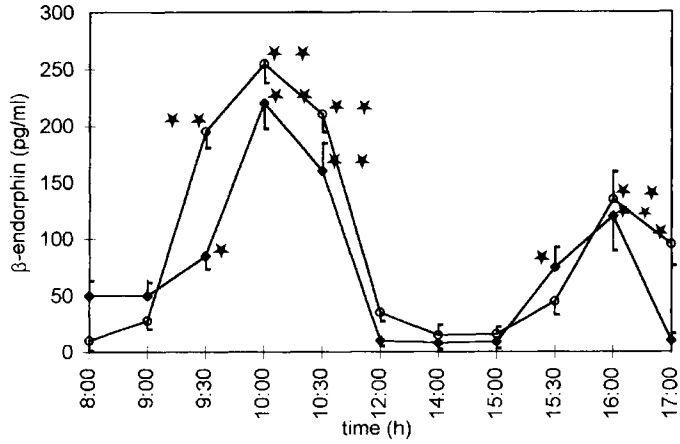

Fig 4. β-endorphin levels (mean with standard error of the mean for four pigs per treatment) after injection with metomidate or ketamine (09.00 and 15.00 h) (* = $P < 0.05$; ** = $P < 0.005$, with respect to the baseline). —o— metomidate; —◆— ketamine.

high muscular activity and screamed. This was not the case after the metomidate injection, where awakening began about 2 h after injection. The awakening process was less difficult for metomidate, as indicated by the lower heart rate ($P < 0.05$).

DISCUSSION

The diurnal rhythm of cortisol levels observed in this study, with higher levels during the night and morning than in the afternoon and evening, was the same as that reported by Becker et al (1985) and Dalin et al (1993). This variation in cortisol levels must be taken into account when cortisol is used as an indicator of welfare or of response to treatment (Broom and Johnson, 1993). Since a normal circadian rhythm was observed, there is no evidence that the blood sampling as such affected plasma hormone levels, nor were they affected by the injection technique (with or without a catheter). After injection with ketamine, a fivefold increase was observed in the

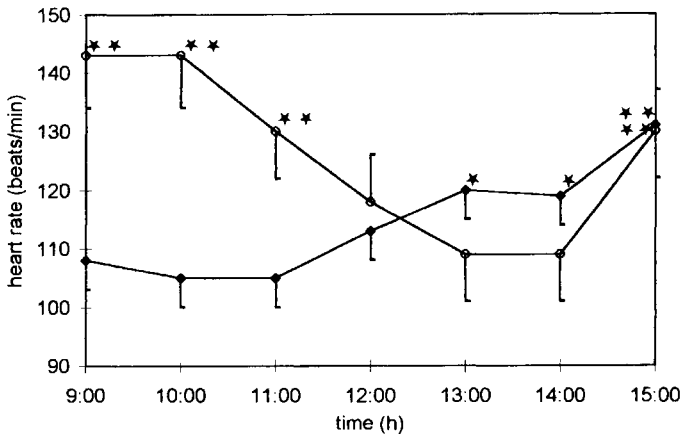

Fig 5. Mean heart rate (mean values with standard error of the mean for six pigs per treatment) after injection with metomidate or ketamine at 09.00 h (* = $P < 0.05$; ** = $P < 0.005$, with respect to the baseline). —◆— metomidate; —o— ketamine.

plasma cortisol levels. This result is in agreement with Oyama et al (1970). Preziosi and Vacca (1988) reported that the injection of etomidate in rats and humans induced ACTH release which was not accompanied by an increase in cortisol levels. This was explained by the strong adrenal suppression capability of etomidate, with 11-β-hydroxylase as the most sensitive target enzyme. Our results suggested a similar but less severe effect of metomidate on the pig adrenal cortex. We could not measure ACTH, however, and thus we have no direct evidence for (a) lower stimulation of pituitary adrenocortical activity, or (b) an impaired response of the adrenal cortex to ACTH. The increases in cortisol and β-endorphin were comparable after the ketamine injection . This provided some evidence for a pituitary-adrenal dissociation after metomidate, since β-endorphin release was at least as great as with ketamine, but the cortisol response was much lower.

Further research should show that there is no difference between the effects of etomidate and ketamine on ACTH release, since β-endorphin release was stimulated by both metomidate and by ketamine. The stimulation of β-endorphin production (May et al, 1991) and the suppression of cortisol (Korte et al, 1992) might explain the lowering of the heart rate after metomidate anaesthesia (fig 5). Heart rate variability, however, can also be explained by differences in the awakening process. In human studies, hallucinations and psychomotoric activities have been described after ketamine anaesthesia (Green, 1979), which may also be related to the increase in heart rate. A lowering of the heart rate may be beneficial when working with pigs that are susceptible to malignant hyperthermia.

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