WELFARE IMPLICATIONS OF THE GAS STUNNING OF PIGS 2. STRESS OF INDUCTION OF ANAESTHESIA

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Abstract *Animal Welfare 1996, 5:* 71-78

The severity of respiratory distress occurring prior to loss of posture during exposure to: 20, 30, 40, 50, 60, 70, 80 or 90 per cent carbon dioxide in air; 2 or 5 per cent residual oxygen in argon; 30 per cent carbon dioxide in argon with either 2 or 5 per cent residual oxygen; or 40 per cent carbon dioxide in argon with either 2 or 5 per cent residual oxygen, was subjectively determined in pigs from their behaviour. The results indicated that exposure to 2 per cent oxygen in argon (anoxia) induced minimal respiratory distress, 30 per cent carbon dioxide in argon with 2 per cent residual oxygen induced a moderate distress and exposure to all the concentrations of carbon dioxide in air induced severe respiratory distress in the pigs. From the animal welfare point of view, using 2 per cent oxygen in argon (anoxia) appears to be the optimum choice for gas stunning pigs. Secondly, a mixture of 30 per cent carbon dioxide in argon with 2 per cent residual oxygen is preferred to 90 per cent carbon dioxide in air.

Keywords: *animal welfare, argon, aversion, carbon dioxide, inhalation, pigs, stunning*

Introduction

From the animal welfare point of view carbon dioxide stunning of pigs is a controversial issue in Europe. Some animal welfarists believe that, as carbon dioxide is an acidic gas, it is pungent to inhale and causes a sense of breathlessness, whereas, others claim that it is an acceptable method. Although a previous publication indicated that carbon dioxide can be pungent and induce breathlessness in humans (Gregory *et al* 1990), some authorities feel that carbon dioxide might not exert similar effects in pigs. But the results of a previous study, in which the initial reaction of pigs was examined during exposure to either 90 per cent argon (anoxia), 30 per cent carbon dioxide in air or 90 per cent carbon dioxide in air, clearly indicated that the pigs did not perceive any aversion to 90 per cent argon, that the majority of the pigs (75%) tolerated any unpleasantness associated with the inhalation of 30 per cent carbon dioxide in air, and the majority of the pigs (88%) showed profound aversion to the presence of 90 per cent carbon dioxide in air (Raj & Gregory 1995). It was also found that the pigs refused to accept a reward (apple) even after 24h of fasting in an atmosphere containing 90 per cent carbon dioxide. This aversive effect of inhalation of a high concentration of carbon dioxide was attributed to the pungency of this gas during initial exposure and/or breathlessness on prolonged inhalation.

It is also important to determine the severity and duration of discomfort caused by the inhalation of a high concentration of carbon dioxide, that occurs prior to loss of consciousness during stunning with this gas. Hoenderken *et al* (1979) thought that the induction of carbon dioxide anaesthesia could take up to 45s. However, Forslid (1991, 1992) suggested that the onset of unconsciousness occurred in about 20 and 15s after exposure to 80 and 90 per cent carbon dioxide respectively, and by implication the procedure was acceptable. Nevertheless, it would still mean that pigs would have to suffer the discomfort caused by the inhalation of this gas for a minimum of 15s. This study aimed at determining the severity and duration of distress caused by the inhalation of stunning gases during the induction of anaesthesia.

Materials and methods

Two separate experiments were conducted in this study. In the first experiment, six groups of six pigs weighing between 15 and 31kg were exposed to each of the carbon dioxide concentrations: 20, 30, 40, 50, 60 and 70 per cent carbon dioxide. The presence of respiratory distress prior to loss of posture (as an indicator of the onset of unconsciousness) was assessed. In the case of 20 and 30 per cent carbon dioxide levels, the pigs were immersed in the gas for one minute and the respiratory distress occurring during that minute was determined. In the second experiment, five pigs weighing 60 to 80kg were exposed to each of the treatment gases, except 50 per cent carbon dioxide in air in which four pigs were used. The treatment gases were: 40, 50, 60, 70, 80 or 90 per cent carbon dioxide in air; 2 or 5 per cent residual oxygen in argon; or a mixture of either 30 or 40 per cent carbon dioxide in argon with either 2 or 5 per cent residual oxygen. The extent of respiratory distress occurring prior to loss of posture, during exposure to these gases was determined.

In both experiments the pigs were placed individually on to a lift, which was then lowered within five seconds into a well containing the gas or gas mixture at a predetermined concentration. In order to determine the effect of handling and lowering the pigs into the well, a day prior to each experiment each pig was handled similarly and lowered into the well containing atmospheric air. On both occasions, the behaviour of the pigs was recorded audiovisually on a videotape, and subsequently subjectively analysed to determine the distress caused by the treatment gases prior to loss of posture. The parameters used to determine the aversiveness of the gases were:

1) Escape attempts: these were defined as occurring when a pig raised its forelegs on the side of the well either prior to, or at the time it was losing its posture.

2a) Respiratory distress occurring during exposure: the cumulative respiratory distress occurring up to the time to loss of posture was subjectively determined by four assessors for all the pigs from the audiovisual recordings. Each assessor had been trained in physiology and they viewed the video recording separately and rated the respiratory distress (respiration score) using a four point scale: 0 = normal ventilation, 1 = mild hyperventilation, 2 = moderate hyperventilation, and 3 = severe hyperventilation.

2b) In addition, the time to the onset of audibly heavier breathing was also recorded.

3) Time to loss of posture: the time to loss of posture (recumbent state), which is considered as a behavioural indicator of the onset of unconsciousness, was

determined from the time the lift reached the bottom of the well using a stopwatch while viewing the videotapes.

The number of animals which showed escape attempts in each treatment gas, the assessors mean respiration score and the time to onset of audibly heavier breathing, and the time to loss of posture were noted for each pig.

The time to the onset of heavier breathing indicated the onset of respiratory distress in that treatment. The duration of the respiratory distress could be calculated from the time to onset of respiratory distress and time to loss of posture. The mean respiration score indicated the extent of distress occurring prior to loss of posture.

It was thought that using these four subjective parameters it should be possible to determine the time to onset, duration and severity of respiratory distress during the induction of anaesthesia with the treatment gases, and help to assess their relative merits on welfare grounds.

The respiration score, time to onset of audibly heavier breathing and time to loss of posture for each pig were subjected to an analysis of variance test to determine the significance of differences between the treatment gases.

Results

The results of experiment 1 showed (Table 1) that isolation from the others and caging of a pig, could act as an aversive stimulus as it resulted in three out of 36 piglets (8%) exhibiting escape attempts whilst they were exposed to air. One out of six piglets (16%) exhibited escape attempts during exposure to 40 and 50 per cent carbon dioxide, and none of the piglets showed escape attempts during exposure to 60 or 70 per cent of this gas. Irrespective of the carbon dioxide concentration, all the piglets showed hyperventilation during exposure to this gas. The subjective assessment of the severity of hyperventilation indicated that in 20 and 30 per cent carbon dioxide levels it was initially low, but increased as the exposure time progressed. The mean respiration scores for 20, 30 and 40 per cent carbon dioxide levels were very similar (more than mild hyperventilation) and these means were also significantly lower than those found with 50, 60 and 70 per cent carbon dioxide ($P < 0.001$). The mean respiration score was similar in 50 per cent or more of carbon dioxide concentrations (moderate to severe hyperventilation). The time to the onset of hyperventilation, as indicated by the mean time to onset of audibly heavier breathing, was very similar in all the carbon dioxide concentrations tested in this experiment. None of the piglets exposed to 20 or 30 per cent carbon dioxide exhibited loss of posture during the one minute exposure time. Loss of posture occurred sooner as the carbon dioxide concentration increased from 40 to 70 per cent, however, it took twice as long with the 40 per cent carbon dioxide when compared with 50 per cent or more of this gas ($P < 0.001$).

The results of experiment 2 are presented in Table 2. Among the 59 pigs used in the second experiment, none of them showed escape attempts during exposure to air. Exposure to 40 to 70 per cent carbon dioxide in air resulted in 74 per cent of the pigs exhibiting escape attempts, and they occurred either prior to loss of posture or coincided with the loss of posture. No escape attempts were observed during exposure to either 80 or 90 per cent carbon dioxide in air. Exposure to 5 per cent residual oxygen in argon resulted in neither escape attempts nor loss of posture during a one minute observation period.

Table 1 **Number of piglets which showed signs of escape and/or breathlessness in experiment 1.**

Treatments	Number of piglets		Respiration score		Time (second) to audibly heavy breathing		Time (second to loss of posture)	
	exposed	attempted escape						
			mean	SD	mean	SD	mean	SD
Air	36	3	0.0	-	-	-	-	-
Carbon dioxide in air:								
20%	6	none	1.7[a]	0.3	5.1	2.6	didn't occur	-
30%	6	none	1.7[a]	0.3	6.9	1.3	didn't occur	-
40%	6	1	1.5[a]	0.2	4.7	0.9	44[b]	11
50%	6	1	2.6[b]	0.4	4.3	4.4	20[a]	5
60%	6	none	2.7[b]	0.3	3.7	3.7	22[a]	4
70%	6	none	2.9[b]	0.2	4.3	4.2	16[a]	4
Statistical significance:			$P < 0.001$ (df = 35)		not significant (df = 35)		$P < 0.001$ (df = 22)	

Means in a column without a common superscript differ significantly.

SD – standard deviation

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Table 2 Number of piglets which showed signs of escape and/or breathlessness in experiment 2.

Treatments	Number of piglets		Respiration score		Time (second) to audibly heavy breathing		Time (second) to loss of posture	
	exposed	attempted escape						
			mean	SD	mean	SD	mean	SD
Air	59	0	0.38^{a}	0.3	10^{a}	7.0	didn't occur	
Carbon dioxide in air:								
40%	5	5	2.4^{cd}	0.3	8^{b}	1.7	38^{bd}	-
50%	4	3	2.3^{cd}	0.7	6^{ab}	1.5	34^{bd}	8.5
60%	5	4	2.1^{cd}	0.3	5^{ab}	0.8	25^{bcd}	2.3
70%	5	2	1.9^{bc}	0.8	3^{a}	1.9	17^{abc}	3.8
80%	5	0	2.1^{cd}	0.2	6^{ab}	2.6	22^{bde}	6.4
90%	5	0	2.4^{cd}	0.1	3^{a}	1.4	15^{ad}	3.3
Argon in air with residual oxygen of:								
20%	5	0	0.6^{a}	0.6	5^{a}	4.1	35^{c}	12.7
5%	5	0	1.1^{b}	0.6	15^{c}	8.7	didn't occur	
30% carbon dioxide in argon with residual oxygen of:								
2%	5	0	1.8^{b}	0.7	6^{ab}	2.2	24^{bd}	7.8
5%	5	0	2.6^{d}	0.2	7^{ab}	3.5	47^{f}	10.8
40% carbon dioxide in argon with residual oxygen of:								
2%	5	1	2.4^{cd}	0.4	7^{ab}	2.7	25^{be}	2.8
5%	5	1	2.2^{cd}	0.6	9^{b}	2.9	32^{bc}	9.4
Statistical significance:			$P<0.001$ (df = 59)		$P<0.001$ (df = 59)		$P<0.001$ (df = 44)	

Means in a column without a common superscript differ significantly.
SD – standard deviation

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No escape attempts were seen during exposure to 2 per cent residual oxygen in argon nor to a mixture of 30 per cent carbon dioxide in argon with 2 or 5 per cent residual oxygen. When the carbon dioxide level in the argon mixture was set at 40 per cent, one out of five pigs showed escape attempts irrespective of the residual oxygen levels (either 2 or 5% oxygen).

The mean respiration score recorded during exposure to air or 2 per cent residual oxygen in argon was low (less than a mild hyperventilation) and these two mean values were not significantly different. Exposure to 5 per cent residual oxygen in argon or to 30 per cent carbon dioxide in argon with 2 per cent residual oxygen resulted in similar mean respiration scores (mild to moderate hyperventilation), and these means were higher than that recorded for either air control or 2 per cent oxygen in argon alone ($P < 0.001$). All the other treatments used in this experiment had significantly higher mean respiration scores, representing moderate to severe hyperventilation ($P < 0.001$). This implies that the degree of hyperventilation during exposure to all concentrations of carbon dioxide in air or to 30 per cent carbon dioxide in argon with 5 per cent residual oxygen, or 40 per cent carbon dioxide in argon with either 2 or 5 per cent residual oxygen were very similar; only 2 per cent oxygen in argon (anoxia) resulted in a low respiration score, similar to that for air.

There were statistically significant differences between the treatments in the time to the onset of audibly heavier breathing, and between 40 and 70 per cent carbon dioxide there was a trend for the mean time to the onset of this effect to decrease as the concentration of carbon dioxide in the atmosphere increased. The mean time to the onset of audibly heavier breathing was similar in concentrations between 40 and 60 per cent of carbon dioxide, 2 per cent oxygen in argon and all the carbon dioxide-argon mixtures. The mean time to the onset of audibly heavier breathing during exposure to 5 per cent residual oxygen in argon was significantly longer.

During exposure to 40, 50 and 60 per cent carbon dioxide, it was possible to determine the time to loss of posture only for one, two and three pigs respectively, because of the severe escape attempts exhibited by the pigs. For the other treatments, it was possible to determine the time to loss of posture for all the pigs. In general, exposure to 40 to 60 per cent carbon dioxide, 2 per cent residual oxygen in argon, 30 per cent carbon dioxide in argon with 2 per cent residual oxygen, or 40 per cent carbon dioxide in argon with either 2 or 5 per cent residual oxygen resulted in a similar time to loss of posture. Exposure to 90 per cent carbon dioxide, in comparison with all the other treatments, resulted in a relatively faster the onset of loss of posture. Exposure to 30 per cent carbon dioxide in argon with 5 per cent residual oxygen resulted in the slowest time to loss of posture ($P < 0.001$).

Discussion

Overall, the results showed that exposure to carbon dioxide either in air or in argon induced moderate to severe hyperventilation in pigs before the onset of unconsciousness occurred. However, the reaction of the pigs varied with the concentration of carbon dioxide. For example, the hyperventilation lasted longer during exposure to 20 and 30 per cent carbon dioxide in air because loss of posture or unconsciousness did not occur with these concentrations of carbon dioxide. Therefore the duration of the hyperventilation was prolonged. Since none of the pigs showed escape attempts during exposure to these low concentrations of carbon dioxide, it can be assumed that the respiratory distress induced with

these low concentrations of carbon dioxide was probably tolerable to the pigs. This assumption is supported by the results of a previous experiment which indicated that the majority of pigs (75%) entered a feeding chamber containing 30 per cent carbon dioxide in air to obtain a reward (Raj & Gregory 1995). Exposure to 40 to 70 per cent carbon dioxide in air induced severe respiratory distress and a considerable proportion of the pigs tried to escape from the gas. The aversive effect of carbon dioxide appears to be profound at the 40 per cent level. This interpretation was also supported by the observation that one out of five pigs showed escape attempts when the carbon dioxide level was set at 40 per cent whilst using the carbon dioxide-argon mixture. Whereas, none of the pigs showed escape attempts during exposure to either 2 or 5 per cent residual oxygen in argon (anoxia alone).

By contrast, exposure to 80 and 90 per cent carbon dioxide in air, though it induced severe respiratory distress, resulted in none of the pigs showing escape attempts. Our previous study indicated that the majority of the pigs (88%) avoided an atmosphere containing this concentration of carbon dioxide, and that the aversive effect of this gas when applied at 80 per cent concentration or higher overwhelmed the motivation to feed after a 24h fast (Raj & Gregory 1995). In the present study, since the loss of posture occurred relatively quickly with these high concentrations of carbon dioxide, it seems likely that the pigs did not have time to show escape attempts because of the rapid the onset of unconsciousness.

The time to onset of audibly heavier breathing and the time to loss of posture decreased as the concentration of carbon dioxide in the stunning atmosphere increased. By subtracting the time to the onset of audibly heavier breathing from the time to loss of posture one can calculate the duration of respiratory distress. This also decreased as the concentration of carbon dioxide increased, from 30s for 40 per cent carbon dioxide to 12s for 90 per cent carbon dioxide. The intensity of respiratory distress, as assessed with the respiration score, appeared to be similar for all the carbon dioxide concentrations.

Exposure to 5 per cent residual oxygen in argon resulted in neither escape attempts nor loss of posture during a one minute exposure time, but induced mild respiratory distress as indicated by the respiration score. This treatment would not be suitable on its own for stunning pigs commercially. However, the 2 per cent residual oxygen in argon treatment induced very little respiratory distress and the time to loss of posture achieved with this treatment was comparable to that achieved with 50 per cent carbon dioxide in air. It can be argued that the cumulative distress caused by the less than mild hyperventilation for 30s, induced in some pigs with the inhalation of argon, is more acceptable on humanitarian grounds than the distress caused by the severe hyperventilation for 12s occurring during the inhalation of 90 per cent carbon dioxide in air. Indeed, our previous study indicated that none of the pigs perceived any aversion to enter into a feeding chamber containing 2 per cent oxygen in argon and some of them lost their posture while feeding (Raj & Gregory 1995). These observations clearly indicated that the inhalation of argon is not aversive and does not induce any sense of breathlessness provided the residual oxygen is maintained at less than 2 per cent.

A mixture of 30 per cent carbon dioxide in argon with 2 per cent residual oxygen induced a slightly less than moderate hyperventilation. However, it reduced the time to loss of posture by 11s when compared with the 2 per cent oxygen in argon treatment. This implies that the addition of 30 per cent carbon dioxide to 60 per cent argon (with 2% residual

oxygen) could induce a rapid loss of consciousness when compared with anoxia alone. In fact, the mean time to loss of posture during exposure to 30 per cent carbon dioxide in argon with 2 per cent residual oxygen was similar to that recorded for 80 per cent carbon dioxide in air. The intensity of the respiratory distress induced with the 30 per cent carbon dioxide in argon with 2 per cent residual oxygen, was also less than that induced with any of the concentrations of carbon dioxide in air tested in this study.

The results of experiment 1 indicated that the respiratory distress induced with 30 per cent carbon dioxide is probably tolerable because there was no escape attempts and the respiration score was also relatively low. In addition, the results of a previous study showed that the majority of the pigs (75%) did not show any aversion to entering a feeding chamber containing 30 per cent carbon dioxide in air (Raj & Gregory 1995). Taken together this implies that a mixture of 30 per cent carbon dioxide in argon with a residual oxygen of 2 per cent is probably not aversive to the pigs, and therefore, could be used for stunning them humanely.

Animal welfare implications

Based on these results, it is proposed that the order of preference of the gases for stunning pigs would be 2 per cent oxygen in argon (anoxia) in which the respiratory distress is minimal but the induction of anaesthesia is relatively slow; followed by 30 per cent carbon dioxide in argon with 2 per cent residual oxygen, which is intermediate with regard to respiration score and rate of induction of anaesthesia; and then 90 per cent carbon dioxide in air in which the induction of anaesthesia is rapid and respiratory distress is severe but short-lasting (about 15s). Stunning with a mixture of a low concentration of carbon dioxide in argon is a new concept, but since it involves a low concentration of carbon dioxide and induces a rapid loss of consciousness it did not appear to impart a sense of breathlessness to the same extent as that perceived with a high concentration of carbon dioxide in air. Therefore it has a welfare advantage over using a high concentration of carbon dioxide alone.

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