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EFFECTS OF AZAPERONE AND ACETYLPROMAZINE ON SOCIAL AND ENVIRONMENTAL BEHAVIOUR IN SHEEP

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1 Acetylpromazine (Acp, 0.05, 0.1 and 0.5 mg/kg) and azaperone (Azp, 1.0, 1.25 and 1.5 mg/kg) were given by intramuscular injection to separate groups of seven familiar Romney ewes. Thirty minutes after injection the sheep were led into a featureless arena and spatial distribution, activity and shade preference were monitored for 20 min by overhead photography.

2 Behavioural response was complex with both stimulant and depressant effects being seen. The response trends with increasing time for all three measures were significantly different for the two drugs. In particular, Azp tended to increase animal dispersion and Acp to decrease it, in agreement with earlier predictions.

3 The dosage range for Azp was adequate for reduction of individual movement and inter-animal distance but steady state effect was not reached in the time period studied. All doses of Azp initially caused disorientation, as measured by shade preference, but this improved as sedation deepened.

4 The highest dose of Acp (0.5 mg/kg) achieved steady state effect on individual movement within the study period. The three doses of Acp caused either no change or a slight increase in inter-animal distance. Disorientation with Acp was less and briefer than that seen with Azp.

Introduction

Phenothiazines, butyrophenones and lithium salts are all antipsychotic drugs used in the treatment of affective disorders. The effects of chlorpromazine (Cpz) and lithium on social behaviour in groups of familiar laboratory rats have been studied (Syme & Syme, 1973; 1974a), but there are no comparable observations for butyrophenone derivatives like azaperone (Azp).

The rationale and methods adopted for the present study follow our previous work with lithium which, like Azp, may have specific anti-aggressive properties. Lithium has been shown to be effective in rats with artificially induced aggression (e.g. footshock, Sheard, 1970) while Azp has been found useful in veterinary practice for preventing fighting in regrouped pigs (Symeons & Van den Brande, 1969). Interpretation of these findings is difficult, however, since these drugs may simply reduce the general environmental awareness of treated animals (Syme & Syme, 1974a).

As noted elsewhere (Syme & Syme, 1974b), experiments with familiar subjects (stable groups) allow differentiation between social and environmental effects of drugs. Animals in stable groups experience only environmental novelty whereas the formation of unstable groups provides an additional confounding source of behavioural influence (social disruption). Such effects are particularly relevant to Azp, since all studies with the drug have involved unstable groups or socially isolated animals; the relative contribution of pharmacological, environmental and social effects to the anti-aggressive drug response has yet to be determined.

When stable groups of rats were observed in an open field, Cpz was found to decrease inter-animal distance (Syme & Syme, 1973) while in another study (Syme & Syme, 1974a), lithium increased distance between animals. Acetylpromazine (Acp), the phenothiazine derivative generally used for veterinary purposes, was therefore chosen for comparison with the effects of Azp on spatial behaviour in sheep. In this species, social behaviour is primarily expressed as allelomimesis (mutual imitation), flocking and vocalization, a repertoire amenable to an experimental paradigm assuming an inverse relationship between

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social status and proximity (McBride, 1971; Syme, Syme, Waite & Pearson, 1975). Interanimal distance in stable groups within a novel 'open field' arena was measured by overhead photography (Syme *et al.*, 1975) and hence only horizontal changes in position were recorded. Such a technique would have limited value for subjects (e.g. pigs) which engage in vigorous and complex social interactions. Azp was expected to resemble lithium and to increase animal dispersion; conversely, in view of our findings with Cpz, one might expect Acp to decrease inter-animal distance.

Ideally, investigations of drug effects on single animals should precede those in the social setting. Consequently a preliminary study (Hughes, Syme & Syme, 1977) compared the response of individual Romney ewes to typical clinical doses of Azp (0.5, 1.0 mg/kg) and Acp (0.1, 0.25 mg/kg) within the 'open field' arena. Whereas Azp (particularly the higher dose) significantly reduced the distance travelled and time spent walking, it increased the numbers of sheep seeen grazing and pawing, and decreased the number heard vocalizing at least once. The only significant effect recorded for Acp was a reduction in the number of sheep heard vocalizing. In that vocalization is a common reaction to social isolation in sheep (Scott, 1945) both drugs appeared to influence 'sociability'. The investigation described here used the same apparatus but with a shaded area so that three measures of response by groups of familiar sheep were obtained from photographs taken above the field: (1) shade preference—a presumed measure of environmental awareness, or disorientation; (2) individual movement—a measure of activity; and (3) individual distance—a measure of 'sociability', or dispersion.

Methods

Forty-nine Romney ewes culled from a common flock of about 250 and aged 2 to 4 years were weighed (50 to 55 kg) and randomly assigned to a control group and six drug groups ($n = 7$) on the day before observation. The sheep used in our earlier study (Hughes *et al.*, 1977) came from the same flock. Spray marker dyes were used to make distinctive signs on the back of each animal to allow identification in the subsequent photographs. The sheep were kept together in a holding yard overnight before testing the following day at 12 h 30 min. The study was conducted outdoors during late spring at the Ruakura Agricultural Research Centre, Hamilton, New Zealand in a featureless arena (14.63 × 14.63 m) surrounded by corrugated iron walls, 2.13 m high. Observations were made after mid-day so the animals were able to use a shaded area covering 11 segments of the field by the SW and NW walls (i.e. the outer squares on two sides of the field). In diagonally opposite corners there were hinged gates through which the animals entered and left the field. The floor was covered with an 8 cm layer of coarse wood shavings on top of an 8 cm layer of pumice sand. Before testing any of the groups, a white plastic grid dividing the arena into 36 × 2.44 m squares was placed on the floor, photographed and then removed. The image on the resulting negative was then transferred to a clear plastic film which, when placed over the test photographs, enabled accurate positioning of subjects.

Each drug group received one of three doses of Azp (1.0, 1.25, 1.5 mg/kg; Stresnil injection, Ethnor) or Acp (0.05, 0.1, 0.5 mg/kg; Boots); the control was isotonic saline. Since Acp had similar, but not significant, effects to Azp in our study with single sheep (Hughes *et al.*, 1977) a wider dose range was employed for the social situation, the highest dose being somewhat greater than that recommended for the sedation of horses, pigs and sheep (0.05 to 0.1 mg/kg, Boots). Our dosage of Azp ranged from the highest used with individual sheep (1.0 mg/kg) to the lowest found effective for preventing fighting in pigs (1.5 mg/kg). The same injection time (30 min) but a longer test period (20 min) were used, in comparison with our initial investigation. Clinical signs of sedation for both drugs appear within 5 to 10 min of intramuscular injection, with the effects persisting for at least 1 to 2 h. Since social settings tend to accentuate the effects of psychotropic drugs (Kinnard & Watzman, 1966) only the lowest level of the effective anti-aggressive range of Azp was used (Symeons, 1970) to ensure some sort of active spatial distribution of animals in what was essentially a passive social test environment.

Saline and drugs were given by deep intramuscular injection in the thigh (0.2 ml/kg) and subjects within a group were yarded together and left undisturbed. After 30 min, groups were directed into the test arena and 1 min later photographs were taken of the field from a 13.7 m tower situated 2.4 m from the south end of the SE wall and 0.6 m out from the wall, using a Zenith-E camera (prime lens = Helios 58 mm, attachment lens = Marexar ultrawider). The camera was manually operated but attached to a tripod fixture which ensured that all photographs were obtained from the same position. One photograph was taken each minute for 20 min for each group; 140 for the experiment.

The midpoint of the square occupied by the subject's head was used to provide co-ordinate data for the position of each sheep in the 140 photographs. These data served as a computer input which yielded the following information over the test period: (1) aggregate distance between each sheep and all other sheep in the group; (2) average changes in position for each sheep from photograph to photograph, i.e. every minute of the test period; (3) percentage occupation of

shaded area by each sheep (x or y co-ordinate equal to 1) transformed ($y = \arcsin\sqrt{P}$) to normalise error variance. Data were interpreted with analyses of variance using the program MANOVA from the SPSS package (Nie, Hull, Jenkins, Steinbrenner & Bent, 1975) and results were considered significant when $P < 0.01$.

Results

Mean results for all seven treatment groups (1 to 7) and the three measures of behaviour (a to c) are shown in Figure 1 for three time periods, 0 to 6 min (I), 7 to 12 min (II) and 13 to 20 min (III). Since the three measures have differing magnitudes and/or units, the differences in height of histograms between measures a, b and c are of no significance, being merely a reflection of appropriate scaling of means. The complexity of behavioural response is immediately apparent, with time being an important variable. When the data were analysed as two-way analyses of variance tables, significant main and interaction effects (treatment by time) were found in all instances except for main effects in interanimal distance. Consequently the interaction mean sum of squares was used as a conservative estimate of the error variance in subsequent comparisons of treatment and time means. When compared over the full 20 min time period, only four treatments were significantly different from control; 1.25 mg/kg Azp increased individual movement and all three doses of Azp decreased shade preference. Significant effects for orthogonal designs comparing the two drugs and relating response to varying doses of a drug and time, are shown in Table 1 and summarised below:

Interanimal distance

(1) Taking all doses together, Azp increased distance as time increased whereas with Acp it decreased.
(2) Considering Azp, the lowest dose (1 mg/kg) increased distance with time more than the highest (1.5 mg/kg).
(3) Considering Acp, distance decreased with time for 0.05 mg/kg but increased for 0.5 mg/kg. Both these effects were greatest in the intermediate time period and then diminished.

Individual movement

(1) There was decreased movement with time when all seven treatments were considered together; this change was greatest for Acp.
(2) Considering the three time periods together, the intermediate dose of Azp increased movement.

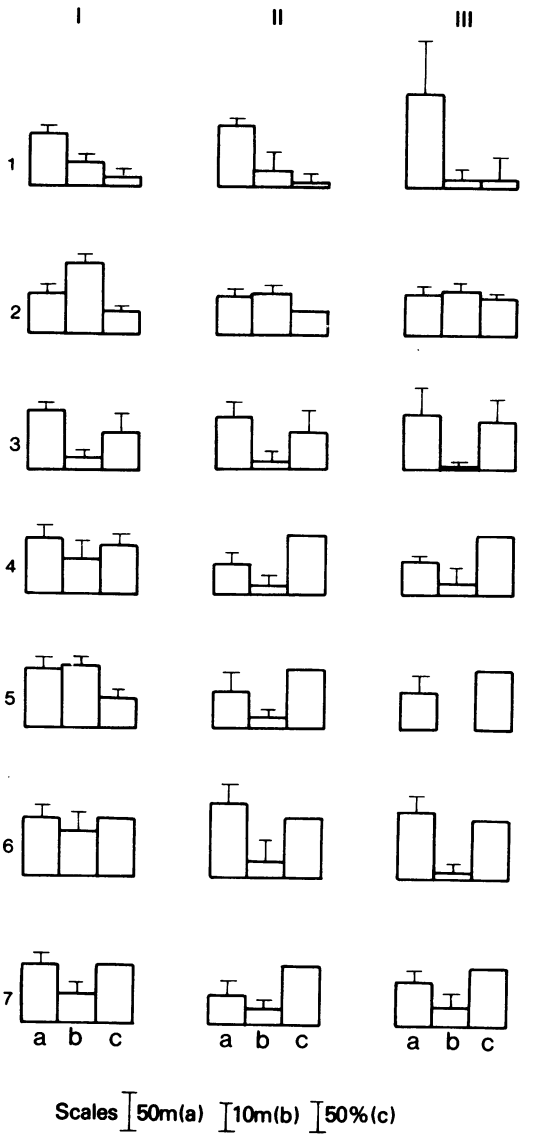

Figure 1 Time and treatment effects on the three measures of behaviour: aggregate inter-animal distance, individual movement and shade preference. Key: 1 to 7 treatments: 1, 2 and 3—azaperone 1.0, 1.25 and 1.5 mg/kg, respectively; 4, 5 and 6—acetylpromazine 0.05, 0.1 and 0.5 mg/kg, respectively; 7—saline control. (a) Inter-animal distance; (b) individual movement; (c) shade preference. I—time 0 to 6 min; II—7 to 12 min; III—13 to 20 min. Error bars shown are standard deviations; where no error bar exists, the error is zero; 100% shade preference (e.g. 7c) was seen in 10 instances.

(3) The intermediate dose of Acp increased individual movement in the first time period and reduced it in the last.
(4) A complicated quadratic-quadratic interaction of Azp dose with time indicated that only 1.25 mg/kg showed a minimum in the intermediate time period and that the effect of dose was greatest in the last time period.

Shade preference

(1) Over all time periods (a) both drugs reduced shade preference compared to the control group (b) the effect of Azp was greater than Acp, and (c) the effect of Azp was greatest at the lowest dose.
(2) Taking all doses together, least shade preference was seen in the intermediate period for Azp and the initial period for Acp.
(3) The intermediate dose of Acp reduced shade preference, but only in the first time period.

Discussion

The stimulant and depressant effects seen with both drugs in this study make an unequivocal interpretation of the data difficult. However, biphasic responses were not totally unexpected since it is known that neuroleptics, while producing a behavioural response of CNS depression, are electrophysiological stimulants (Domino, 1971). Jones (1972) has warned that disturbances in the first 30 min after intramuscular injection of Azp in pigs can cause excitement, and also found that preknockdown excitation usually occurred with intramuscular Acp in horses.

Over the full 20 min test period only four treatments out of a total of 18 (six drug treatments of three measures) were significantly different from control. With such a low proportion of significant effects it might be asked whether the drugs, in the dosages and times selected, displayed any reproducible activity at all. Since the 1% probability level was used in hypothesis testing, it is most unlikely that the effects reported as significant would have occurred by chance alone. Furthermore the results in Table 1 show consistent differences in response to Azp and Acp for all three behavioural measures, and definite response trends were seen for different doses of both drugs.

With the interanimal distance measure in the present setting, the dominant social response is for animals to flock together, especially as the environment becomes familiar with time. Neither drug produced a response which was significantly different from control at any time period. However, there were significant linear time trends for different doses of Azp; distance was significantly increased with time for 1 mg/kg but decreased for 1.5 mg/kg over the same period. This change from a positive to negative effect suggests that inadequate time was allowed for onset of Azp action in the doses used. Acp seemed to act faster than Azp; the quadratic time effect suggesting that steady state effects were reached within the study period. With this behavioural measure, the dose level of Azp, although small in range, was probably adequate. For Acp it may have been too low since the two lower doses were similar to controls and only the highest dose exceeded the response threshold and produced stimulation. Harthoorn (1975) also found that large doses of tranquillizers were needed to modify stable modes of behaviour in small groups of wild animals. The increased dispersion at the highest dose of Acp may represent the only true antisocial effect observed in this study, as all others (Azp) were

Table 1 Summary of significant contrasts for the three measures of behaviour

Measure	*Interaction effects*	*Main effects*
Interanimal distance	(1, 2, 3 vs 4, 5, 6) * I, II, III linear	
	1, 2, 3 linear * I, II, III linear	
	4, 5, 6 linear * I, II, III linear	
	4, 5, 6 linear * I, II, III quadratic	
Individual movement	(1, 2, 3 vs 4, 5, 6) * I, II, III linear	1, 2, 3 quadratic
	1, 2, 3 quadratic * I, II, III quadratic	I, II, III linear
	4, 5, 6 quadratic * I, II, III linear	
Shade preference	(1, 2, 3 vs 4, 5, 6) * I, II, III quadratic	1, 2, 3, 4, 5, 6 vs 7
	4, 5, 6 quadratic * I, II, III linear	1, 2, 3, vs 4, 5, 6
		1, 2, 3 linear

Key: 1, 2 and 3—1.0, 1.25 and 1.5 mg/kg azaperone, respectively; 4, 5 and 6—0.05, 0.1 and 0.5 mg/kg acetylpromazine, respectively; I, II and III—time periods 0 to 6, 7 to 12 and 13 to 20 min, respectively. Significance level was $P < 0.01$. Linear refers to response difference between highest and lowest levels of a factor; quadratic means there is curvature in the factor-response relationship.

accompanied by significant degrees of disorientation (decreased shade preference). The significantly different time trends for Azp and Acp were consistent with expectations from the earlier findings with lithium and Cpz, which increased and decreased dispersion respectively.

With individual movement, 1.25 mg/kg Azp induced significant stimulation over all time periods compared to control, again indicating (since the higher dose of 1.5 mg/kg produced less stimulation) that the doses were adequate to cover the range from stimulation to depression. Similarly it would seem that the Acp doses were adequate because with 0.1 mg/kg there was a maximum in movement in the first time period and a minimum at the last. Nevertheless a longer period may be required for steady state response to Azp and Acp in this measure as movement decreased linearly with time for both drugs, the change being significantly greater for Acp. When the activity of individual sheep was observed in this environment (Hughes *et al.* 1977) only Azp had a significant depressant effect, whereas with individual movement in the social setting, Acp had a greater depressant effect than Azp. Social isolation in sheep is a stressful state (Cairns, 1966) and thus Azp may be the more effective neuroleptic in socially disturbed or 'emotional' sheep.

Over the whole experiment, shade preference in the treatment groups was less than the 100% response seen in control animals, and this effect was greater for Azp compared to Acp. The increased preference with increasing dose of Azp suggests that the lowest dose is acting at the first phase of a biphasic phenomenon. In this measure it is obvious that higher Azp doses, expected to produce the second depressant phase, could not increase preference beyond the maximum control value of 100%. Both Azp and Acp showed evidence of producing steady state responses within the study period, so that within the dosage ranges used, the reduced disorientation for Acp compared to Azp would seem to be a pharmacological property rather than the result of different kinetics.

Thus the complicated response pattern for different doses of the two drugs considered at different times over all three behavioural measures in familiar sheep is best understood in the context of biphasic dose-response curves after allowance for kinetic effects. Stimulant effects at low doses, manifested as increased dispersion and movement and decreased shade preference, become depressant effects (decreased dispersion and movement and increased shade preference) at higher doses. Subtle differences in the dose-response curves and kinetics of the two drugs are apparent. The most evident difference between the drugs at the dosages used in this study is that Acp induces far less disorientation than Azp.

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