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Reduced resident time and pharmacodynamic effects of acepromazine after subclinical multiple dosage in exercised thoroughbreds

C. C. CHOU
C. L. CHEN
B. L. RICE &
P. T. COLAHAN

Department of Large Animal Clinical Sciences, College of Veterinary Medicine, The University of Florida, Gainesville, FL, USA

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C.C. Chou, Department of Veterinary Medicine, College of Veterinary Medicine, National Chung-Hsing University, Taichung, Taiwan. E-mail: ccchou@nchu.edu.tw

Acepromazine (ACP) is a phenothiazine tranquilizer/sedative commonly used in equine veterinary medicine. The tranquilizing/sedating effect induced by ACP produces only limited loss of alertness and co-ordinated motor response of the horses (Gross & Booth, 1995). Therefore, it is usually used in low doses to reduce stress during transportation prior to racing or show events (Smith *et al.*, 1996). On the other hand, because of its tranquilizing effects without appreciably affecting on co-ordination, ACP may be used to obtain better control of excitable horses at the racetrack or show arena and is thus prohibited in competition by most racing commissions and show horse organizations.

In order to reduce ACP in post-race drug test-positive, ACP has been strategically administered by repeated lower doses in the hope that it would reduce the withdrawal time of the drug while maintaining the desirable tranquilizing effect in the racehorses. However, the advantage of such practice is not clear. Whether ACP at this dosage level maintains its beneficial pharmacological effects is unknown. In addition, exercise usually alters the pharmacokinetic course and pharmacodynamics of a drug (Ma, 1990). In a previous study, we have reported an exercise-associated reduction of urinary ACP resident time (Chou *et al.*, 1998) after a single 25-mg ACP injection (single dosage). In this study, we proposed to study the elimination of five daily doses of 5-mg ACP (multiple dosage) in exercise-conditioned/exercised horses and compared with the single dosage regimes. Furthermore, while doses of ACP in precompetitive horses were at subclinical levels (< 0.05 mg/kg), previous studies on the pharmacodynamic effects of ACP (Parry & Anderson, 1983; Robertson, 1987; Freestone *et al.*, 1991; Kim *et al.*, 1994) were mostly by doses above their clinically effective levels (0.15–0.3 mg/kg). In order to assess the effects of ACP at subclinical levels and to determine whether exercise would affect ACP-induced pharmacodynamic responses, the pharmacodynamic effects of a single 25-mg ACP dose or 5-mg ACP for 5 days to horses that have been subjected to standardized exercise were studied.

Horses were trained and assigned to treatment groups as previously described (Chou *et al.*, 1998). Briefly, 12 Thoroughbreds (five mares, five geldings, two stallions) weighing 430–540 kg were preconditioned for 1 month by galloping on a high-speed treadmill until they were fit to gallop 1 mile in 2 min without signs of undue stress. Horses were randomly assigned to four equal groups and rotated among four experimental treatments using a crossover design. The four treatments were: no exercise/no ACP; exercise/no ACP; no exercise/ACP; and exercise/ACP. The drug was administered 10 min after exercise to avoid possible drug effects on locomotion and co-ordination. A single 25-mg dose or five daily 5-mg doses of ACP maleate (PromAce, Fort Dodge, IA, USA) were given i.m. in the dorsal neck musculature. Samples for ACP determinations were obtained at specified times over an 8-day period commencing on the first day (single dosage) or the fifth day (multiple dosage) after the drug administration. Serum samples were collected by jugular venipuncture at 10, 15, 20, and 30 min and 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h. Urine samples were collected on a free catch basis at 0, 2, 4, 8, 12, 24, 48, 72, 96, 120 and 192 h. Serum and urinary ACP concentrations were determined by a one-step enzyme linked immunosorbent assay (Chou *et al.*, 1998). The resident time of ACP in horses was defined by the time at which the immunoreactive ACP concentration was not statistically different from the concentration that was obtained from untreated horses at that particular time, which should be distinct from the mean residence time (*MRT*).

A total of 25 physiological parameters including haematological and hormonal parameters, as well as the electrolyte panel, were measured to assess pharmacological and the pharmacodynamic effects of ACP and/or exercise. Plasma sodium, potassium, calcium, chloride, and carbon dioxide concentration as well as a complete blood count including haematocrit and haemoglobin values were analyzed in the clinical pathology lab of the College of Veterinary Medicine, University of Florida. Serum insulin, cortisol and ACTH concentrations were determined by radioimmunoassay (Coat-a-count, Diagnostic Products Corp., Los

Angeles, CA, USA). Serum glucose value was determined by an enzymatic UV test kit (Sigma Diagnostics, St Louis, MO, USA). Plasma samples at 0, 10 and 30 min and 1, 4, 8 and 12 h were collected for analyses of electrolytes and haematological parameters. Time matched serum samples were used for hormone studies. Glucose and haematocrit levels were monitored for up to 5 days. The main and interaction effects of ACP administration, exercise, and time after drug treatment were analyzed by ANOVA tables. Statistical significance ($P < 0.05$) of these effects was determined by an F-test with pairwise comparisons of treatment combinations conducted at each sampling time point, using Turkey's Honestly Significant Difference procedure (Zar, 1984).

After the change of dosage from a single 25-mg of ACP to multiple 5-mg doses, serum ACP resident time was significantly reduced by 21 h (48 vs. 27 h) when the horses were subjected to prior exercise. For horses that were not exercised prior to ACP administration, the serum ACP resident time was not statistically different between the two dosage regimes (Table 1). In the urine, ACP resident time was significantly decreased by more than 30% (from 112 to 72 h and from 65 to 43 h) in horses receiving multiple dosage regardless of the exercise status (Table 2). Both exercise and multiple dosage contributed to the reduction of ACP resident time (Fig. 1). Overall, horses with multiple dosage and exercise resulted in the shortest serum and urinary resident time.

It has been reported that exercise could increase the elimination of basic drugs (Ma, 1990) because of the lowered pH of body fluids after exercise and the subsequent urine acidifying effects that decrease the renal re-absorption of the drug (Poortmans *et al.*, 1991; Suzuki & Ikawa, 1991). Sporadic examination of urine pH indicated that, in agreement with the possible mechanism, the urinary pH dropped from an average of 8 in nonexercised horses to 5.5 in exercised horses. However, because the extent of urine acidification was not regularly evaluated, we could not correlate urinary pH to the shortened ACP resident time. It should be noted that exercise did not affect serum ACP resident time after single 25-mg dose. It is possible that in view of the lipophilic nature of the drug and a large volume of distribution (Marroum *et al.*, 1994), a tissue reservoir of ACP that, in response to the increased elimination of ACP due to exercise, re-equilibrate the drug from reservoir into blood stream. This reservoir was much smaller in horses receiving multiple low doses because drug administration was accompanied by partial elimination of the drug each day. In the case where the elimination of ACP was not facilitated by exercise, this reservoir effect may also explain why multiple dosage exhibited a similar resident time to single dosage.

Although ACP resident time was greatly reduced by 5 mg repeated daily dose and applied exercise, serum and urinary ACP (peak) concentrations were also significantly decreased from those of single dosage (Fig. 1). As a result, horses receiving 5 mg of ACP for 5 days showed no statistical difference in all tested physiologic parameters when compared with untreated horses. Alternatively, haematocrit (HCT), haemoglobin (HGB), red blood cell (RBC), white blood cell (WBC), neutrophil (NEU) and basophil (BASO) counts were significantly decreased for 1–8 h (Fig. 2) following the administration of a single dose of 25-mg ACP. Standardized treadmill exercise counteracts most of the pharmacodynamic effects of the single 25-mg ACP injection by

Table 1. Serum ACP (mean ± SEM) peak concentration, peak time and resident time after a 25-mg single dosage or five 5-mg multiple dosage of ACP to exercised and nonexercised Thoroughbreds ($n = 12$)

Treatment	Peak conc. (ng/mL)	Peak time (min)	Resident time (h)	
Exercised				
25 mg × 1*	13.8 ± 2.3†	17.3 ± 2.2†	48.0 ± 6.2	
5 mg × 5	3.4 ± 0.4‡		20.9 ± 4.5	26.7 ± 3.9†‡
Non-exercised				
25 mg × 1*	10.5 ± 1.2	36.7 ± 6.4	50.0 ± 7.2	
5 mg × 5	3.3 ± 0.3‡		25.8 ± 6.2	57.6 ± 6.1

*Chou *et al.* (1998). Reprint with permission.
†Significantly different ($P < 0.05$) from the nonexercised group of the same dosage regime,
‡Significantly different ($P < 0.05$) from the single-dosage regime.

Table 2. Urinary ACP (mean ± SEM) peak concentration, peak time and resident time after a 25-mg single dosage or five 5-mg multiple dosage of ACP to exercised and nonexercised Thoroughbreds ($n = 12$)

Treatment	Peak conc. (ng/mL)	Peak time (h)	Resident time (h)	
Exercised				
25 mg × 1*	51.5 ± 4.6	4.5 ± 0.5	64.8 ± 6.8†	
5 mg × 5	27.5 ± 2.6‡		4.4 ± 0.6	43.2 ± 6.6†‡
Non-exercised				
25 mg × 1*	49.2 ± 2.5	4.0 ± 0.0	111.6 ± 12.0	
5 mg × 5	31.6 ± 3.7‡		3.7 ± 0.2	72.0 ± 11.9‡

*Chou *et al.* (1998). Reprint with permission.
†Significantly different ($P < 0.05$) from the nonexercised group of the same dosage regime,
‡Significantly different ($P < 0.05$) from the single-dosage regime.

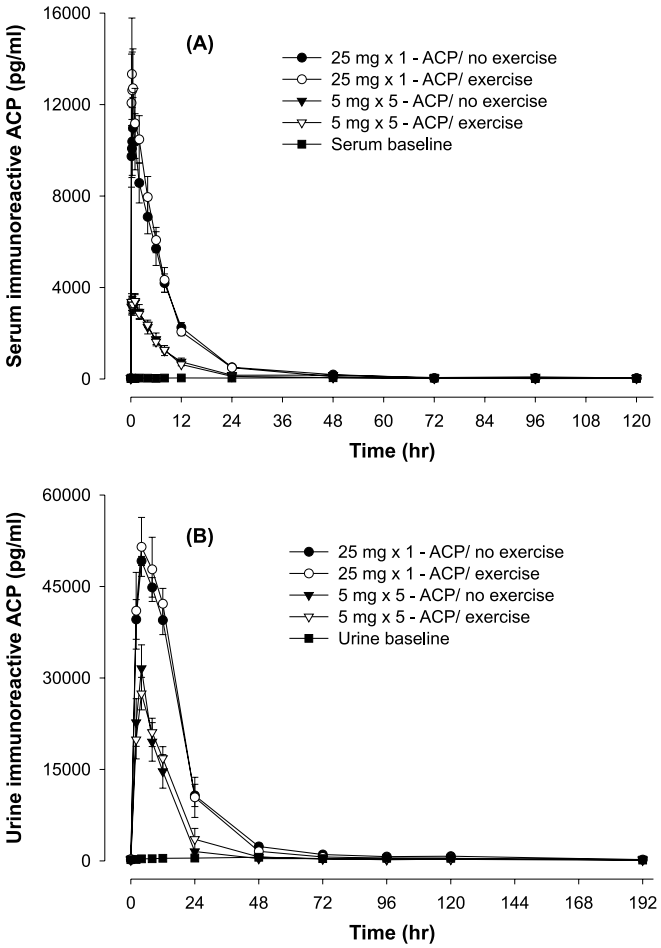

Fig. 1. Serum (A) and urinary (B) ACP immunoreactivity in Thoroughbreds at various times after a single i.m. injection of 25 mg (Chou *et al.*, 1998; reprint with permission) or five daily injections of 5 mg of ACP maleate. Data points represent mean ± SEM for 12 horses.

inducing a significant increase in WBC, NEU, RBC, HCT, HGB, counts and serum ACTH and cortisol for 20 min to 8 h (Fig. 2). As a result, when the horses receiving single dosage were also exercised, the effect of ACP on WBC, NEU and RBC became nonsignificant. This counteraction might be the result of the sympathomimic nature of exercise that possesses effects opposite to the α-adrenergic blockage effects of ACP on peripheral systems. Haematocrit is the most sensitive indicator for the effect of ACP; an i.v. dose of 10 μg/kg ACP (about 5 mg/500 kg) could significantly change the HCT value in the horse (Wood *et al.*, 1992). In this study, HCT was significantly reduced by as much as 30% over control horses for 8 h following single 25 mg dose. The reduction was still significant for 4 h when the horses were exercised. Horses receiving multiple dosage failed to show statistical significance in this measure (Fig. 3). Nevertheless, it should be noted that although the individual pharmacodynamic effects of multiple dosage were not statistically different from the control, it greatly reduced the duration and the statistical significance of the pharmacodynamic effects of exercise (Fig. 2 vs. Fig. 3). The parameters that failed to show significant changes ($P > 0.05$) include: MCV, RDW, MCH, MCHC, platelet, MPV, basophil, eosinophil, monocyte, lymphocyte, CO_2, Ca^{++}, Cl^-, K^+, Na^+, insulin, and glucose concentration.

In conclusion, these results suggest that horses subjected to exercise eliminated ACP more efficiently than nonexercised horses. Change of dosage from a 25-mg ACP single i.m. injection to five 5-mg multiple i.m. injections significantly reduced urinary ACP resident time (and hence withdrawal time) but not serum ACP resident time unless the horses were subjected to prior exercise. Depending on the time the postrace test was performed, it is possible to reduce ACP postrace positive rate if the fifth multiple dosage was given at a time the single dosage was normally given. However, based on the selected endpoints, the expected pharmacodynamic effects of ACP may be largely

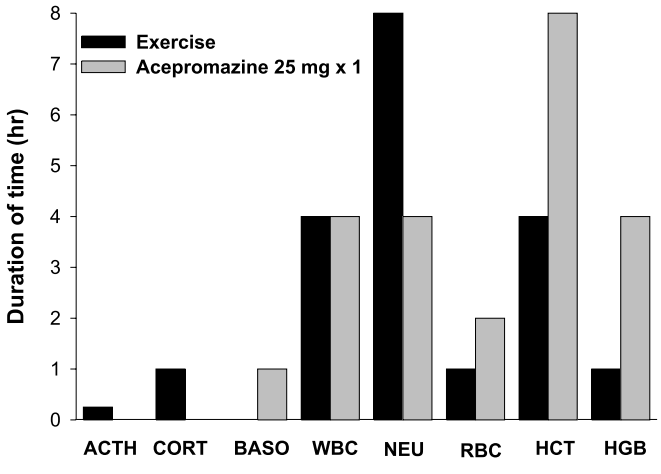

Fig. 2. The duration of pharmacodynamic parameters that are significantly increased by exercise (black bars) or decreased by a single i.m. dose of 25-mg ACP (gray bars) ($n = 12$). HCT, hematocrit; HGB haemoglobin; NEU, neutrophil; RBC, red blood cell count; WBC, white blood cell count.

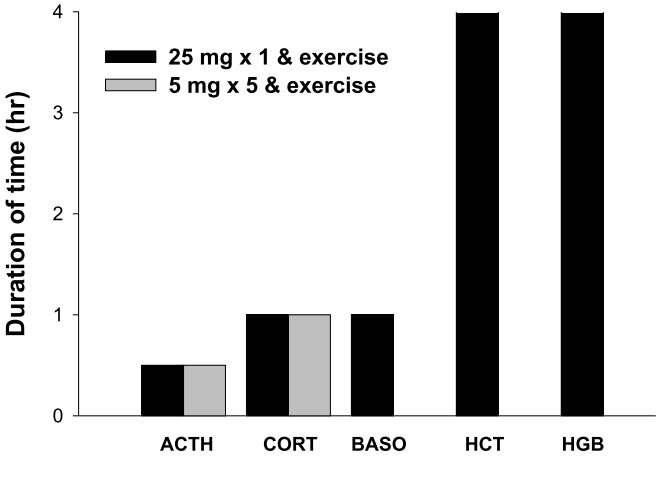

Fig. 3. The duration of pharmacodynamic effects that are significantly changed after ACP administration (25 mg × 1 or 5 mg × 5) in exercised Thoroughbreds. In comparison with Fig. 2, NEU, WBC and RBC were not shown because the opposing effects of exercise and ACP resulted in insignificant changes of these three parameters. ($n = 12$). Abbreviations and notes: See Fig. 2.

diminished when the drug is administered by repeated small doses. Further investigations on the concentrations of ACP in the central nervous system and its affinity to dopaminergic receptors in the horse brain may be beneficial to the understanding of ACP actions at subclinical dosage level.

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