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Levodopa and Aspirin Pretreatment Beneficial in Experimental Decompression Sickness (41322)

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Abstract. Two hundred and two adult, female Sprague–Dawley rats (215 ± 11 g) were compressed to 100 psi (95% N_2 and 5% O_2) for 30 min and decompressed in 3 min. Forty percent of the control animals developed severe clinical signs of decompression sickness (DS) while 31% of the control animals died. Pretreatment with levodopa (4 days at 10 mg/kg ip) or aspirin (30 days at 55 mg/kg in drinking water) decreased the occurrence of DS to 28 or 22%, respectively, while mortality was decreased to 16 or 12%. When levodopa and aspirin were given as a combination pretreatment the incidence of DS was decreased to 11% and mortality to 5.6% only. The increased beneficial effect of combination pretreatment (levodopa and aspirin) suggests two different protective mechanisms of the used drugs.

The pathophysiology of decompression sickness (DS) is not well understood, though clinical signs of this condition were described by Robert Boyle three centuries ago (1). Today, it is speculated that the primary cause of DS is formation of gas bubbles in blood and other tissues (2–4). The clinical manifestations of fast decompression are unpredictable. Hematologic and humoral alterations are observed already in the early phases of blood–bubble interaction (5). The early symptoms lead usually to more severe clinical manifestations of DS. Widespread ischemia occurs, platelet survival is altered (6–9), neurological changes are observed (10), and in the most severe cases damage to the spinal cord or to the brain develops.

Rational therapy for DS has not been established. Pharmacological agents that might help prevention or treatment of DS are still sought (1). Discovery of a therapeutic agent beneficial in treatment or in prevention of DS would be of great practical importance since rapid recompression is the only successful therapy. However, compression chambers are not always available and any appreciable delay in the treatment of DS leads to failure. Therefore, it is speculated that pretreatment with choice agents prior to platelet changes and irreversible damage might be beneficial.

Sprague–Dawley female rats were used in the work. DS was introduced after 30 min compression and rapid decompression, with a mortality rate of 31% in the control animals. The experimental animals were pretreated with levodopa, a catecholamine that seems to be beneficial in recovery from paralysis induced by air embolism (12) and in decreasing coagulation (13), or with aspirin that affects platelet functions.

Materials and Methods. *Animals.* Two hundred and two adult, female Sprague–Dawley rats weighing 215 ± 11 (SD) g (9–11 weeks old) were used in the experiments. All animals were housed individually and were given food and water *ad libitum*. Eighteen hours before decompression, the food was removed. The fasted, unanesthetized animals were weighed and placed in individual, perforated Plexiglas cages (5 × 7 × 5 in.) with 10 cages contained in a Plexiglas cage unit. The unit containing rats was placed in a noiseless Vacudyne animal hyperbaric chamber (0.5 m^3) and the chamber prepared for a decompression (diving) regime.

Pressurization. The Vacudyne animal hyperbaric chamber with the animals was pressurized to 30 psi with compressed air at a rate of 6 psi/min. At the chamber pressure of 30 psi the compressed gas mixture was changed to 95% nitrogen/5% oxygen until 100 psi (at a rate of 10 psi/min). Thus the elapsed time of pressurization from 0 to 30 psi was 5 min and for pressurization from 30 to 100 psi an additional 7 min. Upon reaching a chamber pressure of 100 psi, a ventilation rate of 10 liters/min (with a driving

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pressure of 120 psi) was established and maintained throughout the duration of the compression (30 min). Chamber pressure was constant throughout the compression. Opening of a valve induced decompression at a rate of 10 psi/15 sec from 100 to 30 psi (2 min, 45 sec). At 30 psi a ball valve was opened permitting a rapid decompression from 30 to 0 psi (15 sec).

All dives were performed between 1 and 4 pm to avoid the effects of circadian variations. Ten animals were randomly chosen by close body weight match for exposure to a compression–decompression profile. The animals in compression chamber were observed through a window. After decompression the housing unit was taken out of the chamber and the undisturbed animals were surveyed for the development of DS symptoms.

Control animals. Forty-eight animals were exposed to the dive without any pretreatment.

Levodopa-pretreated animals. Levodopa (10 mg/kg) was dissolved in warm saline and injected ip. Fifty animals received the drug once per day (at the same hour of the day) for 4 days prior to compression–decompression experiment. The last administration of levodopa was just prior to the dive.

Aspirin-pretreated animals. Aspirin (55 mg), dissolved each day in water (20 ml), was given to individually caged rats. Each animal drank daily 12–16 ml of water and in this way ingested 40–45 mg of aspirin during 24 hr. Though aspirin dissolved in water hydrolyzes very slowly and acquires the odor of acetic acid (14), no such odor was present when water bottles were filled with fresh water.

Pretreatment with levodopa and aspirin. Fifty-four animals in this group received a combination of levodopa and aspirin as pretreatment. The dose for levodopa was chosen because 10 mg/kg of levodopa induces pressor response (15).

For statistical evaluations, contingency tables were prepared and χ^2 and P values were calculated in order to test the association between treatment and decompression sickness. Clinical manifestations of DS in control groups of animals were highly reproducible.

Results. *Control animals.* Forty percent of the animals developed DS (Table I). The mortality rate in this group was 31% (Table II). Death occurred at 6.3 ± 2.3 min after the decompression.

Levodopa-pretreated animals. Twenty-eight percent of the animals in this group developed DS (Table I), while the mortality rate was 16% (Table II). Death of the animals was delayed to 21.2 ± 14.2 min after the decompression. There was a significant difference in mortality rate between this and the control group.

Aspirin-pretreated animals. Twenty-two percent of the rats in this group developed DS after the dive (Table I). The mortality rate was 12%. Death occurred 9.5 ± 4.6 min after the decompression. A significant difference in mortality rate existed between this and the control group.

Pretreatment with levodopa and aspirin. After combination pretreatment 11% of the animals developed DS (Table I). The mortality rate in this group was only 5.6% (Table II). There was a significant difference in mortality rate between this and the control group. The onset of death was delayed to 15.2 ± 6.7 min.

χ^2 and P values obtained from the contingency tables indicate (a) a highly significant difference ($P < 0.01$) of mortality rates

TABLE I. CLINICAL MANIFESTATIONS OF DS IN RATS AFTER RAPID DECOMPRESSION

Group	No. rats	Body weight (g) ± SD	Animals with DS symptoms		Animals with DS (%)
			Paraplegia	Total paralysis followed by death	
Control	48	202 ± 16	4	15	40
Levodopa	50	212 ± 16	6	8	28
Aspirin	50	214 ± 14	5	6	22
Aspirin and levodopa	54	212 ± 15	3	3	11

TABLE II. COMPOSITE STATISTICAL EVALUATION[a] OF DS MORTALITY IN RATS AFTER PRETREATMENT WITH LEVODOPA AND ASPIRIN

Animal group	Mortality rate (%)	Level of significance
Control	31.3	<0.01
Levodopa	16.0	
Control	31.3	<0.01
Aspirin	12.0	
Control	31.3	<0.01
Aspirin and levodopa	5.6	
Levodopa	16.0	NS
Aspirin	12.0	
Levodopa	16.0	<0.05
Aspirin and levodopa	5.6	
Aspirin	12.0	<0.05
Aspirin and levodopa	5.6	

[a] χ^2 test.

between untreated control rats versus levodopa-pretreated animals, (b) a highly significant difference ($P < 0.01$) between untreated control rats versus aspirin-pretreated animals, (c) a highly significant difference ($P < 0.01$) between untreated control rats versus aspirin- and levodopa-pretreated rats, (d) no significant difference between levodopa-pretreated animals versus aspirin-pretreated rats, (e) significant difference ($P < 0.05$) between levodopa-pretreated animals versus animals pretreated with aspirin and levodopa, and (f) significant difference ($P < 0.05$) between aspirin-pretreated rats versus animals pretreated with aspirin and levodopa.

Discussion. Search for a drug that might be used in the effective treatment of DS has not been successful. Furthermore, because DS develops abruptly, such a drug would be of little value if vital organs are damaged. However, a drug that fails in treatment of acute DS may still be successful if used as pretreatment. For this reason our interest was focused on agents that might be effective in pretreatment, decreasing occurrence, severity and mortality of experimentally induced DS.

The blood–bubble interface in DS triggers platelet aggregation and damage to the vascular walls (16–18). The platelets seem to play a key role in the production of endothelial surface lesions (19). Furthermore, dead platelets increase hazards of coagulation in already hemoconcentrated blood of DS victims (20). In this work rats were pretreated with levodopa and with aspirin. Both drugs are clinically used, and their effects are well recognized. Aspirin was chosen as an inhibitor of platelet functions and platelet aggregation (21). Philip and co-workers (22) have shown that aspirin, given shortly before a dive, does not have any effect on occurrence of DS. However, blood changes were less pronounced after decompression when aspirin was used. A single dose of aspirin, given orally 1 hr before the dive, did not prevent occurrence of DS in rats, as Bennett and Brock (23) have shown. In our work pretreatment with aspirin lasted 30 days. Levodopa was used because it is beneficial in Parkinsonism, in decreasing rigidity, in increasing locomotion (24), and as a potent vasoactive drug (25, 26). Levodopa appears to be helpful in recovery from experimental spinal cord injury (27), and in recovery from air embolism-induced paraplegia in rats (12). Our results show that aspirin or levodopa are beneficial when used as pretreatment in decreasing significantly clinical signs of serious forms of DS. When used in combination the effect of both drugs was even more striking. Instead of 31% mortality as in the control animals, the combination treatment decreased occurrence of death to a low value of 5.6%.

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