Effects of Phenformin HCl on Rats Subjected to Simulated High Altitude

JOSEPH P. BUCKLEY, R. JOHN SOLARO, and HERBERT BARRY, III

Abstract □ Phenformin HCl has been reported to protect rats from the lethal effects of hypoxia of high altitude by preventing cardiovascular collapse. Hexamethonium Cl abolished the pressor response of phenformin HCl in cats providing additional evidence that one of the actions of phenformin is possible facilitation of ganglionic activity. The compound increased spontaneous motor activity of animals subjected to a simulated altitude of 6,400.8 m. (21,000 ft.) and produced a slight sedative effect in animals at normal atmospheric pressure. Phenformin HCl, 75 mg./kg., orally, markedly improved the lever-press shock-avoidance performance of rats at altitudes between 5,486.4 m. (18,000 ft.) and 7,315.2 m. (24,000 ft.). Altitudes in excess of 6,400.8 m. produced a much greater detrimental effect on learning than on performance and phenformin did not improve the ability of rats to learn an avoidance program at these elevated altitudes.

Keyphrases □ Phenformin effect, rats—simulated high altitudes □ Respiratory stimulation—elevated altitude hypoxia □ Hexamethonium activity—phenformin effect □ Motor activity, elevated altitudes—phenformin effect □ Learning performance, elevated altitudes—phenformin effect

Investigators have attempted to identify compounds which could protect man from the adverse effects of hypoxia of high altitude. These compounds would not only be advantageous during high-altitude exposure but might also produce beneficial effects in such clinical conditions as diseases of the lungs and cardiovascular system which lead to oxygen deficiency. Compounds capable of inducing systemic acidosis have been reported to produce beneficial effects during hypoxia (1–6). Carbonic anhydrase inhibitors, which attenuate the respiratory alkalosis observed during hypoxia exposure, reduce the symptoms of acute mountain sickness and have been reported to produce other beneficial effects in animal and man subjected to hypoxia (7–10). Selle (11), who reported that the administration of insulin decreases resistance to hypoxia, attributed this effect to the lower blood glucose levels occurring after insulin administration.

Phenformin HCl, an orally effective hypoglycemic agent, has been reported to increase glucose uptake and simultaneously reduce oxygen uptake (12, 13). Williams and Steiner (14) reported that phenformin inhibited certain oxidative enzymes leading to increased anaerobic glycolysis with an increase of lactic acid production and an increase in glucose uptake of muscle tissues. This increase in anaerobic glycolysis would be expected to lower oxygen requirements. Accordingly, Powell and Buckley (15) investigated the actions of phenformin on the lethal effects of hypoxia in rats subjected to a simulated high altitude of 9,144 m. (30,000 ft.). These investigators reported that phenformin HCl protected both anesthetized and unanesthetized animals from the lethal effects of severe hypoxia. Their data indicated that death occurring in the control saline-treated groups of both anesthetized and unanesthetized animals was due to severe central hypoxia accompanied or caused by cardiovascular collapse. Low doses of phenformin HCl (100–800 mcg./kg.) in anesthetized cats produced a stimulation of the superior cervical sympathetic ganglia. Since many investigators (16–18) have reported that resistance to hypoxia is much greater in sympathetic ganglia than in the brain and spinal cord, cardiovascular tone could very well be maintained by peripheral stimulation of autonomic ganglia. Powell and Buckley (15) further reported that even prior to decompressing the animals phenformin HCl exerted a stimulatory effect on the respiratory system which apparently resulted in more complete equilibration of the blood with ambient oxygen and thus could account for the higher P_{O_2} values observed in the drug-treated animals. An important additional effect observed in the phenformin-treated animals prior to decompression on each trial was a decrease in P_{CO_2} which should have permitted the animals to hyperventilate without as severe an increase in blood pH as seen in the controls. The effects of phenformin on blood-gas and acid-base balance were qualitatively similar to those produced by carbonic anhydrase inhibition (9). Electroencephalograms obtained from both anesthetized and unanesthetized rats suggested that the saline-treated animals suffered a more severe depression of the central nervous system than did the phenformin-treated animals (15).

The preliminary data obtained in the authors' previous studies indicate that phenformin HCl protected rats from the lethal effects of hypoxia by preventing cardiovascular collapse and central depression while concomitantly producing marked respiratory stimulation accompanied by arterial P_{CO_2} and pH significantly lower and arterial P_{O_2} significantly higher than that in nontreated animals. This present paper reports on a further study of the mechanism(s) by which phenformin protects experimental animals from hypoxia at simulated altitude, and also reports on the effects of phenformin on performance and learning of rats subjected to simulated high altitudes.

METHODS

Effects of Hexamethonium on the Blood Pressure Response to Phenformin—The action of an autonomic blocking agent on the pressor responses to phenformin HCl was investigated using the conventional ganglionic blocking agent, hexamethonium Cl, with the hypothesis that if ganglionic facilitation is one of the actions of phenformin, sufficient doses of hexamethonium should abolish this response. Cats weighing between 1.9 and 3.0 kg. were anesthetized with sodium pentobarbital, 35 mg./kg., i.p. A midline incision was made into the abdominal cavity, and the renal artery and vein of both kidneys tied off to eliminate the excretion of hexamethonium *via* the kidneys and thereby permit maximum accumulation of the

compound. Blood pressure was recorded from a femoral artery *via* a transducer connected to a polygraph (Grass), and a femoral vein was catheterized to permit the administration of drugs. Preliminary experiments were conducted in four cats to determine the dose of phenformin producing maximal pressor effects by administering cumulative doses of the compound starting with 0.1 mg./kg., i.v., and increasing the dosage logarithmically every 10 min. until a maximum cumulative dose of 25.6 mg./kg. was administered.

In order to test the effects of hexamethonium on the pressor activity of phenformin, successive doses of hexamethonium were administered after elicitation of control responses to 3.2 mg./kg. of phenformin, i.v. The dosage sequence of hexamethonium was 1, 2, 4, 8, 16, and 32 mg./kg. or until the expiration of the animal. The dose of phenformin was repeated after each injection of hexamethonium and the effects on blood pressure recorded with a 15-min. time interval between doses of hexamethonium.

Effects of Phenformin on Spontaneous Motor Activity of Rats Subjected to Simulated High Altitudes—The hypobaric chamber used in these studies was constructed by the Bethlehem Corp. of Bethlehem, Pa., and had inner dimensions of 182.88 cm. (72 in.) wide, 50.8 cm. (20 in.) deep, and 101.6 cm. (40 in.) high. The chamber was designed so that the animals could be decompressed to simulated altitudes of 5,486.4 to 6,705.6 m. (18,000–22,000 ft.) within 4 min. with a constant flow of air of approximately 75 l./min. The chamber included a total of 240 electrical inputs and outputs so that behavioral studies could be programmed and the data recorded. Spontaneous motor activity in the chamber was recorded by two photocell units designed by Furgiuele *et al.* (19). Forty-eight albino rats weighing approximately 200 g. each were given single 1-hr. tests in the chamber; half 3 hr. following oral administration of phenformin (75 mg./kg.) and the other half 3 hr. following saline. The 24 animals in each group were subdivided into three test conditions: simulated altitudes of 4,267.2, 6,400.8, and 8,534.4 m. (14,000, 21,000, and 28,000 ft.). Each time the animals broke the beam a count was automatically registered on a digital counter. The total number of counts was recorded at the end of a 1-hr. period. Four actophotometers (Metro Industries) were used in a separate study to determine the effects of phenformin on spontaneous motor activity of rats at the laboratory's normal atmospheric pressure 335.3 m. (1,100 ft.). Ninety-six albino rats were divided into four groups of 24 animals each and treated with saline, 18.25, 37.5, and 75.0 mg./kg. of phenformin, respectively, *via* oral administration 3 hr. prior to the 1-hr. test.

Effects of Phenformin on Performance and Learning by Rats Subjected to Simulated High Altitudes—Four operant conditioning chambers (Lehigh Valley Electronics, Fogelsville, Pa., model 1316) containing two LVE 1352 levers on the wall were placed into the decompression chamber and connected to the programming and recording equipment outside the chamber. An additional four boxes were placed in an adjacent room to study simultaneously the effects of phenformin at laboratory altitude (335.3 m.). The following general procedure was used in the 10 behavioral studies undertaken. Eight albino Wistar rats weighing approximately 200 g. were tested concurrently, each in a separate operant-conditioning box. Each test box was enclosed in an insulated ventilated cubicle (LVE model 1316C) which minimized any undesirable environmental differences between the four test boxes inside the decompression chamber and the other four boxes outside the chamber. The animals were trained to perform a continuous avoidance of a painful electric shock (300 v. a.c. through 150,000 ohms resistance in a scrambled pattern to the grid floor) with a duration of 0.5 sec. and an interval of 5 sec. between shocks. Each press on the right-hand lever initiated a 20-sec. interval until the next shock, so that the animal received a shock only when it allowed 20 sec. to elapse after the avoidance response. A negative reinforcement was used rather than a positive food reward because of the possibility of hypoxia inhibiting appetite. Psychopharmacological studies of this type of performance have been reviewed by Barry and Buckley (20). The programming equipment consisted of transistorized timing and switching modules (Massey-Dickinson, Inc.), and each shock and each avoidance lever press in each test box were automatically registered on counters and by an event marker. The totals were recorded each 0.5 hr. throughout the 2-hr. session. The four animals in the decompression chamber and the four animals at normal atmospheric pressure were subdivided into two pretreated with phenformin and two with saline, 2.5 to 3 hr. prior to the start of each 2-hr. session. Therefore, the sessions included the range of time between 2.5 and 5 hr. following drug administration.

Table I—Cumulative Pressor Effects of Phenformin in Anesthetized Cats[a]

Cumulative Dose, mg./kg., i.v.[b]	Mean Pressor Effect (mm. Hg ± *SE*)
0.1	1.3 ± 1.3
0.2	5.5 ± 2.6
0.4	9.5 ± 2.8
0.8	6.5 ± 0.6
1.6	16.3 ± 3.9[c]
3.2	21.8 ± 7.7
6.4	14.3 ± 1.5
12.8	0.5 ± 0.5[d]
25.6	0.0

[a] N = 4. [b] Interval between doses, 10 min. [c] $p < 0.05$ when compared to response at previous dose level. [d] $p < 0.01$ when compared to response at previous dose level.

Table II—Effects of Hexamethonium on the Hypertensive Activity of Phenformin[a] in Anesthetized Cats

Animal No.	Wt., kg.	Doses of Hexamethonium, mg./kg.—Mean Increase in Arterial Blood Pressure in mm. Hg: 0	1	2	4	8	16	32
1	1.9	25	25	20	7	0	D[b]	—
2	2.4	30	20	15	4	0	0	D[b]
3	3.0	27	27	22	10	0	D[b]	—
4	2.0	40	30	20	8	0	D[b]	—
$\bar{X}$	2.3	30.5	25.5	19.3	7.3	0	—	—

[a] 3.2 mg./kg., i.v. [b] Death.

RESULTS

Effects of Hexamethonium on the Blood Pressure Response to Phenformin—The effects of the cumulative administration of phenformin HCl in anesthetized cats are summarized in Table I. The cumulative dose producing the maximal pressor effect was 3.2 mg./kg., i.v.; therefore, this dose was utilized in investigating the effects of hexamethonium on the pressor activity of phenformin. A cumulative dose of 8.0 mg./kg. of hexamethonium (Table II) completely eliminated the pressor response to phenformin. This blockade adds further evidence in support of the contention that phenformin facilitates ganglionic transmission.

Effects of Phenformin on Spontaneous Motor Activity of Rats Subjected to Simulated High Altitudes—The average number of counts in the photocell units at the end of the 1-hr. decompression period at 5,486.4 m. was 115 ± 15 for the saline-treated animals and 116 ± 33 for the phenformin-treated animals; at 6,400.8 m., the average counts were 65 ± 21 for the saline-treated and 96 ± 24 for the phenformin-treated animals; at 8,534.4 m., the average counts were 56 ± 24 for the saline-treated and 67 ± 39 for the phenformin-treated animals. The data indicate a progressive decrease in spontaneous activity with increasing altitudes. Phenformin

Table III—Experimental Design for Studies 1 and 2: Determination of Optimal Oral Dose of Phenformin HCl

Group[b]	Training (5–7 sessions)[a] Dose, mg./kg.	Training Altitude, m.	Test 1 (5 sessions) Dose, mg./kg.	Test 1 Altitude, m.	Test 2 (5 sessions) Dose, mg./kg.	Test 2 Altitude, m.
1	0	335.3	0	5486.4	0	335.3
2	0	335.3	37.5	5486.4	37.5	335.3
3	0	335.3	75.0	5486.4	75.0	335.3
4	0	335.3	150.0	5486.4	150.0	335.3
5	0	335.3	0	335.3	0	5486.4
6	0	335.3	37.5	335.3	37.5	5486.4
7	0	335.3	75.0	335.3	75.0	5486.4
8	0	335.3	150.0	335.3	150.0	5486.4

[a] Two hours each. [b] N = four per group.

Table IV—Effects of Oral Administration of Phenformin on Mean Number of Shocks per Minute Received by Experimental Animals in Studies 1 and 2[a]

N	Last Two Training Sessions, $\bar{X} \pm SE$	Dose, mg./kg.	Test 1 (5 Sessions) $\bar{X}_1 \pm SE$	$\bar{X}_1 - \bar{X} \pm SE$	Test 2 (5 Sessions) $\bar{X}_2 \pm SE$	$\bar{X}_2 - \bar{X} \pm SE$
			5,486.4 m.		**335.3 m.**	
4	1.18 ± 0.34	0	1.64 ± 0.11	+0.46 ± 0.34	0.96 ± 0.16	−0.22 ± 0.46
4	1.04 ± 0.41	37.5	1.09 ± 0.33	+0.05 ± 0.12	0.79 ± 0.27	−0.25 ± 0.26
4	0.82 ± 0.111	75.0	0.78 ± 0.07	−0.04 ± 0.14	0.52 ± 0.15	−0.30 ± 0.07
4	1.35 ± 0.45	150.0	2.28 ± 0.40	+0.93 ± 0.62	1.21 ± 0.45	−0.14 ± 0.31
			335.3 m.		**5,486.4 m.**	
4	0.72 ± 0.16	0	0.92 ± 0.40	+0.20 ± 0.30	1.55 ± 0.66	+0.83 ± 0.52
4	1.28 ± 0.33	37.5	1.46 ± 0.26	+0.18 ± 0.24	2.01 ± 0.39	+0.73 ± 0.48
4	1.86 ± 0.66	75.0	1.07 ± 0.38	−0.79 ± 0.79	1.53 ± 0.58	−0.33 ± 0.91
4	1.18 ± 0.35	150.0	0.82 ± 0.23	−0.36 ± 0.14	1.16 ± 0.28	−0.02 ± 0.17

[a] Two-hour sessions.

increased activity by only 1% at 4,267.2 m., by 48% at 6,400.8 m., and by 20% at 8,534.4 m. The maximal beneficial effect of phenformin was at the moderate altitude of 6,400.8 m. When the study was repeated in different photocell units at normal atmospheric pressure, mean counts for the 24 saline-treated animals over a 60-min. period were 589 ± 55 compared to a mean count of 487 ± 67 for the animals receiving 75 mg./kg. of phenformin. The mean counts for the animals receiving 18.25 mg./kg. and 37.5 mg./kg. of phenformin were approximately equal to the saline-treated animals. Therefore, although 75 mg./kg. of phenformin HCl *per os* increases spontaneous motor activity of animals subjected to simulated high altitude (6,400.8 m.), this dose produced slight depression at normal atmospheric pressure.

Effects of Phenformin on Performance and Learning by Rats Subjected to Simulated High Altitudes—The initial study was undertaken to investigate the effects of varying doses of phenformin on the performance of albino Wistar rats subjected to a simulated altitude of 5,486.4 m. The experimental design, outlined in Table III, comprised eight groups, each containing two rats, with the second study representing a replication of the design. Performance at the end of training was compared with test performance for the same animals. High altitude *per se* produced a decrement in performance, shown by a marked increase in the number of shocks received by the saline-treated animals at 5,486.4 m. compared to the animals tested at normal atmospheric pressure (335.3 m.). Phenformin HCl, 75 mg./kg., orally, 2.5 to 3 hr. prior to decompression prevented the detrimental effects of hypoxia on performance (Table IV).

Since 75 mg./kg. of phenformin HCl had previously been shown in this laboratory to be the dose producing maximal protection from the lethal effects of severe hypoxia (15), this dose was used in the next five studies. The overall experimental design was similar to that described in *Methods* and outlined in Table III, with the exception that in Studies 3 to 5 the animals were subjected to a simulated altitude of 5,486.4 m., in Study 6 to a simulated altitude of 7,315.2 m., and in Study 7 to a simulated altitude of 6,400.8 m. The effects of simulated altitudes of 7,315.2 and 6,400.8 m. on performance of the experimental animals are summarized in Table V. The higher altitudes produced greater detrimental effects on performance of the rats than were observed at 5,486.4 m. The most beneficial effect of phenformin HCl occurred at 6,400.8 m., with approximately a five-fold increase in the number of shocks received by the saline-treated animals compared to practically no change in the number of shocks received by the animals treated with phenformin (Table V). These data, however, are not conclusive because of the small number of animals in each experimental group and because of the great variation between groups in the number of shocks during the training session. Table VI summarizes the data obtained in Studies 1 to 7 comparing the effects of phenformin HCl (75 mg./kg., orally) and saline on performance at elevated altitudes ranging between 5,486.4 and 7,315.2 m. Phenformin HCl markedly decreased the detrimental effects of elevated altitudes on performance without affecting performance at normal atmospheric pressure.

Studies 8 to 10 were designed to investigate the effects of elevated altitudes on learning of the continuous avoidance response. The experimental design was identical to that previously described with the exception that half of the animals were trained at the elevated altitude and half trained at normal atmospheric pressure (335.3 m.). The altitudes used were 7,315.2 m. in Study 8, 6,400.8 m. in Study 9, and 5,486.4 m. in Study 10. These elevated altitudes produced marked detrimental effects on the ability of the experimental animals to learn the continuous avoidance response. All of the untrained, phenformin-treated animals died when subjected to the avoidance program at 6,400.8 and 7,315.2 m. and 50% of the saline treated animals died at 7,315.2 m.; the survivors failed to learn the avoidance response program. The mean number of shocks per minute received by rats trained at 5,486.4 and 335.3 m. are summarized in Table VII. The animals trained at 5,486.4 m. received

Table V—Mean Number of Shocks per Minute Received by Rats in Studies 6 and 7

Study	N	Training, $\bar{X}$	Dose of Phenformin, mg./kg.	Altitude, m.	Test, $\bar{X}_1$	$\bar{X}_1 - \bar{X}$
6	2	1.13	0	7315.2	2.54	+1.40
	2	1.65	75	7315.2	2.26	+0.60[a]
	2	0.39	0	335.3	0.42	+0.03
	2	0.66	75	335.3	0.40	−0.25
7	2	0.62	0	6,400.8	3.80	+3.18
	2	2.11	75	6,400.8	2.12	+0.01
	2	1.38	0	335.3	0.80	−0.58
	2	2.51	75	335.3	1.90	−0.61

[a] $p < 0.05$ when compared to mean increase in shocks per minute in placebo-treated animals.

Table VI—Mean Number of Shocks per Minute Received by Rats in Studies 1 to 7

N	Training $\bar{X} \pm SE$[a]	Dose of Phenformin, mg./kg.	Altitude, m.	Test, $\bar{X}_1 \pm SE$[b]	$\bar{X}_1 - \bar{X} \pm SE$
18	1.18 ± 0.15	0	5,486.4–7,315.2	2.07 ± 0.25	+0.89 ± 0.32
18	1.27 ± 0.15	75	5,486.4–7,315.2	1.48 ± 0.19	+0.20 ± 0.08[c]
18	0.98 ± 0.11	0	335.3	0.81 ± 0.06	−0.17 ± 0.07
18	1.32 ± 0.20	75	335.3	1.12 ± 0.17	−0.20 ± 0.06

[a] Mean of last 2-hr. training session. [b] Mean of first 2-hr. test session. [c] $p < 0.05$ when compared to mean increase in shocks per minute in placebo-treated animals.

Table VII—Mean Number of Shocks per Minute Received by Rats Trained at 5,486.4 m. and 335.3 m., Respectively, in Study 10

Altitude, m.	Dose of Phenformin, mg./kg.	N	Training, $\bar{X} \pm SE$	N	Test, $\bar{X}_1 \pm SE$
5,486.4	0	4	2.61 ± 0.11	12	1.18 ± 0.18
5,486.4	75	4	2.20 ± 0.36	12	1.29 ± 0.17
335.3	0	4	0.93 ± 0.19	12	1.32 ± 0.21
335.3	75	4	1.01 ± 0.31	12	1.17 ± 0.16

more than twice the number of shocks per minute as those trained at 335.3 m. These animals appeared to acclimate rapidly to the hypoxic conditions, since in 15 subsequent sessions in which the animals were exposed to different sequences of each experimental condition, the mean number of shocks per minute received by the rats tested at 5,486.4 m. was actually slightly less than the mean number of shocks received by the animals tested at 335.3 m.

DISCUSSION

Data previously obtained in this laboratory indicated that phenformin HCl protected experimental animals from the lethal effects of hypoxia by preventing cardiovascular collapse (15). The mechanism suggested for this effect was a direct or indirect stimulation of autonomic ganglia by the compound, thereby maintaining cardiovascular tone during prolonged decompression. The present data provide additional evidence for this action of phenformin, since hexamethonium abolished the pressor effects of intravenously administered phenformin HCl in anesthetized cats. Since phenformin HCl also produces a concomitant increase in respiration, there is the possibility that the compound maintains the integrity of the cardiovascular system *via* reflexogenic receptors.

Phenformin HCl, 75 mg./kg., orally, markedly improved the lever-press shock-avoidance performance of rats tested at elevated altitudes ranging between 5,486.4 and 7,315.2 m. This dose of the compound also appeared to be superior to 37.5 and 150 mg./kg. in preventing the detrimental effects of high altitude on performance. These data agree with findings previously reported on the ability of phenformin HCl to prevent the lethal effects of hypoxia on anesthetized and unanesthetized rats subjected to marked elevated altitudes. Elevated altitudes, especially at 6,400.8 m. and above, produced a much more detrimental effect on learning than on performance. Naive rats failed to learn at 6,400.8 to 7,315.2 m., and the combination of these two stressors (learning the lever-press shock-avoidance program and hypoxia) proved lethal to a large percentage of the animals. Phenformin HCl did not improve the ability of the experimental animals to learn an avoidance response at high altitude. The compound in a dose of 75 mg./kg., orally, increased the spontaneous activity of animals subjected to a simulated altitude of 6,400.8 m., although the same dose produced a slight sedative effect in animals at normal atmospheric pressure.

At elevated altitudes, phenformin HCl, a compound which prevents the lethal effects of hypoxia, produced a general stimulatory effect, and markedly improved the lever-pressing shock-avoidance performance. On the other hand, the compound did not enhance the ability of the animals to learn the same shock-avoidance response at these elevated altitudes.

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