Sodium Nitroprusside-Induced Hypothermia in Mice

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Abstract □ The hypothermic response following sublethal doses (1.25, 2.5, and 5 mg/kg) of sodium nitroprusside was investigated in mice. The magnitude and duration of rectal temperature depression were shown to be dose related. Oral administration of nitroprusside (5 mg/kg) failed to alter rectal temperature significantly; subcutaneous, intraperitoneal, intravenous, and intracerebral injections at the same dosage level caused respective drops of 3.61, 3.65, 6.44, and 3.48°. The degree of rectal temperature depression following nitroprusside (5 mg/kg ip) was dependent upon the ambient temperature. The time course of the effect of nitroprusside (5 mg/kg ip) on tail temperature was noted. A transient rise in tail temperature, which coincided with the onset of rectal temperature depression, was attributed to the vasodilatory effect of the drug. Tail temperature depression occurred at the peak and throughout the remainder of the rectal temperature response, suggesting that nitroprusside may decrease heat production. Body temperature depression *via* intracerebral administration, as well as pronounced sedation following nitroprusside injection, suggests a central component to the thermolytic response.

Keyphrases □ Sodium nitroprusside—effect on body temperature, various routes of administration, various ambient temperatures, mice □ Temperature, body—effect of sodium nitroprusside, various routes of administration, various ambient temperatures, mice □ Hypothermia—induced by sodium nitroprusside, effect of various routes of administration and various ambient temperatures, mice □ Antihypertensive agents—sodium nitroprusside, effect on body temperature, various routes of administration, various ambient temperatures, mice

In previous experiments (1), sodium nitroprusside (sodium nitroferricyanide) produced marked hypothermia in mice. Rectal temperature depression averaging approximately 3.5° was noted 15 min following sublethal doses (5 mg/kg ip) of this potent hypotensive agent. Although the mechanism of this response has not been established, nitroprusside apparently is devoid of action on central and peripheral cholinoceptive sites.

To provide insight into the mechanism of nitroprusside-elicited hypothermia, this study investigated in greater detail the temperature response to sodium nitroprusside.

EXPERIMENTAL

Adult male Swiss albino mice, 20–25 g, were housed in groups of 25 with *ad libitum* access to laboratory food[1] and water for several days prior to experimentation. For 24 hr prior to and including the time of the experiment, the mice were kept in a room free from drafts at a constant environmental temperature of 23 ± 1° unless otherwise noted.

Solutions of sodium nitroprusside were freshly prepared with distilled water in concentrations (calculated as the salt) such that a volume of 0.01 ml/g was delivered. Intracerebral injections, however, were administered in a fixed volume of 0.01 ml/mouse.

A thermistor thermometer[2] was used for obtaining rectal and tail temperatures. Rectal temperatures were recorded with a thermistor probe inserted to a distance of 2.5 cm and held in position until constant readings were attained. Tail temperatures were obtained by placement of a disk probe on the base of the tail. A small amount of electrode paste was applied to the surface of the disk probe.

Intracerebral injections were accomplished according to the method of Haley and McCormick (2). Sodium nitroprusside or water (warmed to 38°) was administered at a point lateral to the midline joining the anterior bases of the ears. A 22-gauge needle attached to a microliter syringe[3] was inserted through the skull to a depth of 3 mm. The prior injection of several animals with a solution of methylene blue (0.5%) resulted in localization of the dye in the third and fourth ventricles.

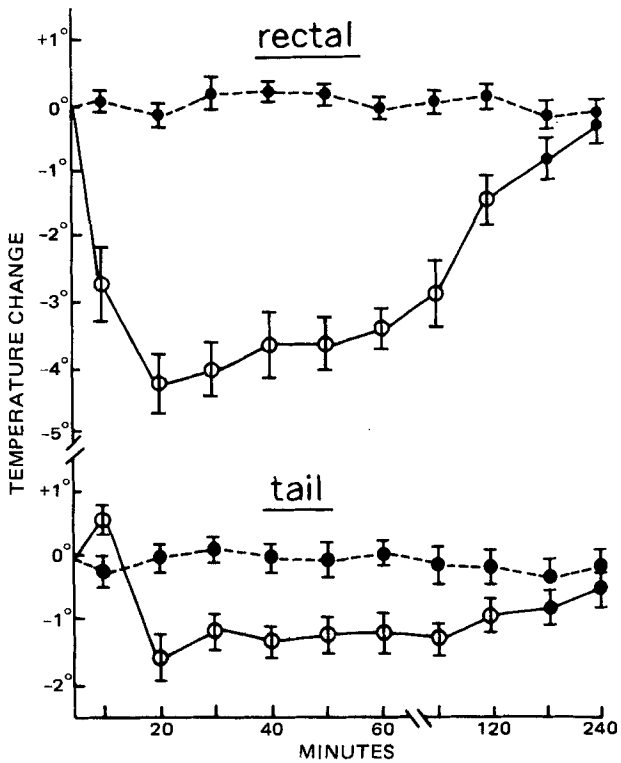

Figure 1—*Time course of the effect of distilled water (0.01 ml/g ip) (---●---) and sodium nitroprusside (5 mg/kg ip) (—●—) on rectal and tail temperature in mice. Water or nitroprusside was administered at zero time. Open symbols denote a significant difference ($p < 0.05$) from the water treatment at the corresponding time interval. Each point represents the average of 12 determinations. Vertical bars represent standard errors.*

At the start of each day's testing, the mice were placed singly in circular wire-mesh cages and individual weights were obtained with a triple-beam balance[4]. Immediately following weight determination, initial temperatures were recorded and treatment administration was accomplished. Temperatures were recorded again at various intervals. Unless otherwise stated, all treatments were given by the intraperitoneal route. Animals that served as controls received distilled water (0.01 ml/g).

In the comparison of mean temperature changes (*i.e.*, the difference between temperature immediately prior to and at the appropriate interval following treatment), statistical significance was determined by the use of the Student *t* test. Temperature differences were considered to be significant at the probability level of 5% or less.

RESULTS AND DISCUSSION

The effect of sodium nitroprusside (1.25, 2.5, and 5 mg/kg ip) on the body temperature of mice was determined at various intervals over 4 hr (Table I). At the lower dosage level (1.25 mg/kg), nitroprusside produced a body temperature depression lasting approximately 30 min. The intensity and duration of the hypothermic response were increased with higher doses. At all dosage levels investigated, maximal hypothermia was evident within 15–30 min. Decreased locomotor activity and muscle

[1] Purina chow.
[2] YSI model 46 Tele-Thermometer, Yellow Springs Instrument Co., Yellow Springs, Ohio.
[3] Hamilton.
[4] Ohaus.

Table I—Effect of Intraperitoneal Administration of Sodium Nitroprusside at Various Dosage Levels on the Body Temperature of Mice

Minutes	Temperature Change (Mean ± *SE*)[a]			
	Water	Sodium Nitroprusside		
		1.25 mg/kg	2.5 mg/kg	5 mg/kg
15	+0.28 ± 0.18°	−1.35 ± 0.25°[b]	−2.43 ± 0.27°[b]	−4.08 ± 0.53°[b]
30	+0.35 ± 0.16°	−0.79 ± 0.19°[b]	−1.45 ± 0.21°[b]	−4.11 ± 0.51°[b]
60	+0.23 ± 0.24°	−0.15 ± 0.09°	−0.93 ± 0.25°[b]	−3.48 ± 0.43°[b]
90	+0.16 ± 0.21°	−0.07 ± 0.19°	−0.73 ± 0.23°[b]	−2.82 ± 0.52°[b]
120	+0.28 ± 0.15°	+0.02 ± 0.20°	+0.18 ± 0.14°	−1.56 ± 0.43°[b]
180	+0.13 ± 0.16°	−0.03 ± 0.18°	+0.19 ± 0.17°	−0.53 ± 0.37°[b]
240	+0.22 ± 0.17°	+0.10 ± 0.14°	+0.24 ± 0.16°	−0.13 ± 0.31°

[a] Temperature changes represent the difference between body temperature recorded initially and that obtained at the designated interval following water or nitroprusside treatment in groups of 12 mice. [b] Compared to water at the corresponding time interval, $p < 0.05$.

Table II—Mean Changes in Body Temperature 30 min following Sodium Nitroprusside Administration by Various Routes in Mice

Route	Water		Sodium Nitroprusside (5 mg/kg)	
	Initial Temperature	Temperature Change (Mean ± *SE*)[a]	Initial Temperature	Temperature Change (Mean ± *SE*)[a]
Oral	37.52°	−0.12 ± 0.16°	37.16°	−0.21 ± 0.29°
Subcutaneous	37.41°	−0.15 ± 0.11°	37.61°	−3.61 ± 0.33°[b]
Intraperitoneal	37.29°	0.00 ± 0.12°	37.45°	−3.65 ± 0.46°[b]
Intravenous	37.46°	+0.24 ± 0.25°	37.60°	−6.44 ± 0.52°[b]
Intracerebral	37.61°	−0.48 ± 0.33°	37.75°	−3.48 ± 0.34°[b]

[a] Temperature changes represent the difference between body temperature recorded initially and that obtained 30 min following water or nitroprusside treatment in groups of 12 mice. [b] Compared to water by the corresponding route, $p < 0.05$.

Table III—Body Temperature Changes 30 min after Intraperitoneal Injection of Sodium Nitroprusside to Mice at Various Ambient Temperatures

Ambient Temperature	Water		Sodium Nitroprusside (5 mg/kg)	
	Initial Temperature	Temperature Change (Mean ± *SE*)[a]	Initial Temperature	Temperature Change (Mean ± *SE*)[a]
18°	36.95°	+0.06 ± 0.09°	37.11°	−6.53 ± 0.46°[b,c]
23°	37.33°	+0.06 ± 0.16°	37.52°	−4.44 ± 0.44°[b]
28°	38.06°	−0.01 ± 0.10°	38.24°	−1.75 ± 0.14°[b,c]

[a] Temperature changes represent the difference between body temperature recorded initially and that obtained 30 min following water or nitroprusside treatment in groups of 12 mice. [b] Compared to water at the corresponding ambient temperature, $p < 0.05$. [c] Compared to sodium nitroprusside at 23°, $p < 0.05$.

weakness developed approximately 10 min after administration and were present throughout the entire body temperature response. Visible shivering and increased muscle tone were unapparent during and upon recovery from hypothermia.

Sodium nitroprusside (5 mg/kg) was administered to groups of 12 mice by the oral, subcutaneous, intraperitoneal, intravenous, and intracerebral routes, and the effect on body temperature at 30 min was noted (Table II). The compound was inactive when given by oral intubation. This observation supports the work of Page *et al.* (3), who noted that sodium nitroprusside produced minimal hypotension by this route in various species. Significant hypothermia occurred following injection by the subcutaneous, intraperitoneal, intravenous, and intracerebral routes. Greatest activity was seen with the intravenous route; rectal temperatures averaged 6–7° lower than the values obtained prior to treatment.

Symptoms were depressant in nature, except in the case of the intracerebral route where the animals exhibited profound clonic and tonic convulsant activity after a latent period of approximately 30 min. During this latency, however, symptoms were characteristically depressant. All animals injected by this route died between 60 and 90 min following treatment. Intracerebral injection with lower doses of nitroprusside (0.025 mg/kg) caused a lesser degree of hypothermia (0.81°) and no incidence of lethality (data not included).

The influence of ambient temperature upon the thermal response of mice to sodium nitroprusside (5 mg/kg ip) is illustrated in Table III. Animals exposed to an environmental temperature of 18° exhibited rectal temperature depression amounting to 6.53°, while mice exposed to 23 and 28° gave drops of 4.44 and 1.75°, respectively. These values were significantly different from one another.

Figure 1 depicts the time course of changes in rectal and tail temperatures after sodium nitroprusside (5 mg/kg) administration. Prior to the intraperitoneal injection, rectal and tail temperatures averaged 37.50 and 25.12°, respectively. The rectal temperature response was similar to that shown in Table I. Tail temperature was elevated (+0.54°) 10 min after nitroprusside injection, followed by a sharp decline lasting approximately 3 hr.

These results support the earlier report (1) that sodium nitroprusside induces a pronounced fall in the body temperature of mice, dependent upon the dose and route of administration.

The ambient temperature had a pronounced effect on the intensity of body temperature depression by nitroprusside. Since the metabolism rate is temperature dependent, it is important to regulate the hypothermic response while measuring other pharmacological parameters. Such information may be useful in future studies on the mechanism of action of this compound.

During the rapid fall in rectal temperature, tail temperature was transiently elevated (Fig. 1). Presumably, this elevation was due to the well-known vasodilator action of the drug. Vasodilation and, thus, increased peripheral heat loss apparently contribute early in the production of hypothermia. However, from 20 min onward, tail temperature depression paralleled the rectal temperature response. This observation suggests that a mechanism (or mechanisms) other than vasodilation contributes to the thermolytic response. Tail temperature depression throughout most of the rectal temperature response suggests that decreased heat production, rather than increased heat dissipation, plays a major role in nitroprusside-induced hypothermia. The vasodilatory action of the drug may, in fact, play only a minor transient role in body temperature depression.

Thus far, no data support an effect of nitroprusside on the central nervous system (CNS), nor is there any evidence that its effects are mediated through this system. Certain observations during this investigation suggest a possible central component of the hypothermic activity of nitroprusside. The decrease in body temperature produced by intracerebral administration and the pronounced sedation produced by doses that

significantly depress body temperature suggest that nitroprusside causes hypothermia, in part, through an action on the CNS. Conceivably, the drug may cause nonspecific depression of thermoregulatory control centrally. This action could explain the absence of compensatory shivering and increased muscle tone, although nitroprusside interference at the effector level could account for these absences.

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Application of ^{13}C-NMR Spectroscopy to *In Vitro* Analysis of Enzyme Kinetics

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Abstract □ The conversion of D,L-α-^{13}C-histidine to similarly labeled α-^{13}C-histamine by bacterial and mammalian histidine decarboxylase was studied by ^{13}C-NMR spectroscopy and GLC–mass spectrometry. The results obtained with the partially purified bacterial enzyme were in essentially perfect agreement with results obtained simultaneously with a standard radioisotopic method using carboxyl-labeled-^{14}C-L-histidine. For a crude tissue preparation of the mammalian enzyme, the radioisotopic method indicated an activity three times that based on ^{13}C-NMR measurement of α-^{13}C-histamine. The difference in results was accountable in terms of additional ^{13}C-NMR signals attributable to products other than histamine due in part to enzymatic degradation of the latter.

Keyphrases □ D,L-α-^{13}C-Histidine—conversion to histamine by histidine decarboxylase, ^{13}C-NMR and GLC–mass spectral study □ D,L-α-^{13}C-Histamine—conversion from histidine by histidine decarboxylase, ^{13}C-NMR and GLC–mass spectral study □ Enzyme activity—histidine decarboxylase, conversion of D,L-α-^{13}C-histidine to histamine, ^{13}C-NMR and GLC–mass spectral study □ ^{13}C-NMR spectroscopy—study of conversion of D,L-α-^{13}C-histidine to histamine by histidine decarboxylase □ GLC–mass spectrometry—study of conversion of D,L-α-^{13}C-histidine to histamine by histidine decarboxylase

Many *in vitro* assays of enzyme activity are relatively inconvenient, time consuming, or indirect in that measurements are based on cofactors or coproducts rather than the major product. Furthermore, compounds are frequently assayed for inhibitory activity using crude tissue preparations where the primary product may be exposed to further enzymatic transformations that may or may not be affected by the test compound. An example is the assay of specific histidine decarboxylase (EC 4.1.1.22) in minced tissue or crude extracts of mammalian tissues (1). One method of assay measures the ^{14}C-carbon dioxide evolved from carboxyl-labeled ^{14}C-histidine (2), and other methods are based on the measurement of the histamine produced (1).

The increasing availability of stable isotopic intermediates suggested exploration of NMR spectra using synthetic isotopic-enriched substrates as a convenient means of quantitatively and directly determining the kinetics of formation of multiple products in a crude enzyme assay. For initial exploration, ^{13}C-enriched histidine (3) was used; ^{13}C-NMR results were compared with a standard radioisotopic method (2) using histidine decarboxylase of both bacterial and mammalian origin. With the bacterial enzyme, mass spectrometry served as an additional comparison.

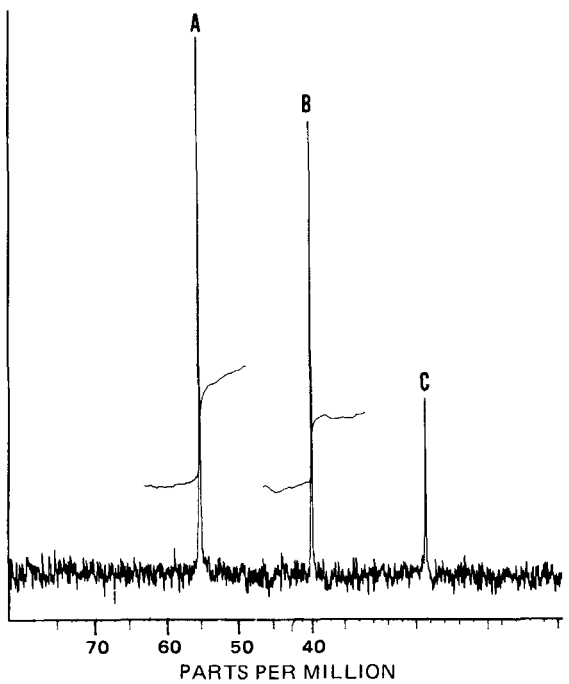

Figure 1—*^{13}C-NMR spectrum of bacterial histidine decarboxylase reaction mixture [conversion of D,L-α-^{13}C-histidine (M + 4) to α-^{13}C-histamine (M + 4)]. Protein was removed by heat precipitation. Key: A, α-^{13}C-histidine; B, α-^{13}C-histamine; and C, reference ^{13}C-acetate.*

EXPERIMENTAL

Carboxyl-labeled-^{14}C-L-histidine (13 mCi/mmole) was obtained commercially[1]. Nonradioactive isotopic D,L-histidine (2,5-^{2}H,3-^{15}N,α-^{13}C-labeled) was synthesized with 80% enrichment in M + 4 and 20% M

[1] New England Nuclear Corp., Boston, Mass.