Naloxone does not alter the “regulated” decrease in core temperature during hypoxemia in guinea pigs

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Crisanti, Kim C., and James E. Fewell Naloxone does not alter the “regulated” decrease in core temperature during hypoxemia in guinea pigs. J. Appl. Physiol. 85(3): 1150–1159, 1998.—In newborns and adults of a number of species, exposure to acute hypoxemia produces a “regulated” decrease in core temperature, the mechanism of which is unknown. The present experiments were carried out in chronically instrumented newborn (5–10 days of age; n = 59) and older (25–30 days of age; n = 61) guinea pigs to test the hypothesis that the endogenous opioids mediate this regulated decrease in core temperature. During an experiment, core temperature, oxygen consumption, and selected ambient temperature were measured in a thermocline (linear temperature gradient of 10–40°C) during a control period of normoxemia, an experimental period of normoxemia or hypoxemia (inspired oxygen fraction 0.10), and during a recovery period of normoxemia following an intraperitoneal injection of naloxone hydrochloride (a nonspecific opioid antagonist; 1, 2, or 4 mg/kg) or vehicle. Naloxone did not significantly alter basal core temperature or the core temperature response to acute hypoxemia in newborn or older guinea pigs. Naloxone did, however, decrease basal oxygen consumption in newborn and older guinea pigs and altered the thermoregulatory effector mechanism used to decrease core temperature during hypoxemia in the newborn guinea pigs. Our data do not support the hypothesis that the endogenous opioids mediate the regulated decrease in core temperature that occurs in newborn and older guinea pigs during exposure to acute hypoxemia.

METHODS

One hundred and twenty Hartley strain guinea pigs were studied. Each pup, born by spontaneous vaginal delivery, was housed with its mother and siblings (22 ± 1°C, 20–30% relative humidity, and 12:12-h light-dark cycle). Although 22°C is below the reported thermoneutral zone of newborn guinea pigs (12), each pup had the opportunity to select its ambient temperature between experiments by huddling with its siblings and/or mother (i.e., behavioral thermoregulation).

Surgical preparation. Each guinea pig underwent one operation before the study. Within 2–3 days of an experiment, each pup was anesthetized by inhalation of halothane (2.0% for induction and for maintenance) in oxygen. A paramedian laparotomy was done, and a battery-operated biotelemetry device (PhysioTel TA10ETA-F20, Data Sciences International) was inserted into the peritoneal cavity for later measurement of core temperature. After surgery, the pups were returned to their mother for recovery.

All surgical and experimental procedures were carried out in accordance with the Guide to the Care and Use of Experimental Animals, provided by the Canadian Council on Animal Care, and with the approval of the Animal Care Committee of the University of Calgary.

Experimental protocol. For an experiment, each pup was removed from its mother and siblings, weighed, and placed in a thermocline into which flowed room air for a period of 1–2 h. At the end of this stabilization period, measurements were made during a control period. A period of five consecutive measurements at 2-min intervals in which core temperature was stable (±0.2°C) was considered to be a suitable control period. After control measurements, the guinea pig was removed from the thermocline and given an intraperitoneal (ip) injection of naloxone hydrochloride at doses of 1, 2, or 4 mg/kg or an equal volume of vehicle. Next, the animal was returned to the thermocline and monitored for an additional 30 min. Each animal then underwent a 60-min experimental...
Fig. 1. Effect of normoxemia (left) and hypoxemia (right) on core temperature ($T_c$) in newborn guinea pigs after an intraperitoneal (ip) injection of saline (A) and changes ($\Delta$) in $T_c$ from control after an ip injection of saline or after 1, 2, (B) or 4 mg/kg of naloxone (C). C, control; I, injection; N, experimental period (normoxemia); H, experimental period (hypoxemia); R, recovery period. *$P < 0.05$ vs. C; #$P < 0.05$ vs. response observed with saline.
Fig. 2. Effect of normoxemia (left) and hypoxemia (right) on $T_c$ in older guinea pigs after an ip injection of saline (A) and changes in $T_c$ from control after an ip injection of saline or 1, 2 (B) or 4 mg/kg of naloxone (C). *P < 0.05 vs. C.
period of exposure to either normoxemia or to acute hypoxemia. After the experimental period, each animal underwent a 60-min recovery period of normoxemia. Dependent variables were recorded at 6-min intervals during the experimental and recovery periods.

Two age groups of animals were studied. A newborn group of 59 guinea pigs was studied between 5 and 10 days of age, and an older group of 61 guinea pigs was studied between 25 and 30 days of age. The animals in each age group were randomly assigned to one of eight experimental groups. Experimental group I received an ip injection of vehicle after the control period and experienced normoxemia during the experimental period (newborn guinea pigs n = 5; older guinea pigs n = 8); experimental group II received an ip injection of vehicle after the control period and experienced hypoxemia during the experimental period (newborn n = 8; older n = 8); experimental group III received an ip injection of naloxone (1 mg/kg) after the control period and experienced normoxemia during the experimental period (newborn n = 5; older n = 8); experimental group IV received an ip injection of naloxone (1 mg/kg) after the control period and experienced hypoxemia during the experimental period (newborn n = 8; older n = 8); experimental group V received an ip injection of naloxone (2 mg/kg) after the control period and experienced normoxemia during the experimental period (newborn n = 8; older n = 8); experimental group VI received an ip injection of naloxone (2 mg/kg) after the control period and experienced hypoxemia during the experimental period (newborn n = 8; older n = 8); experimental group VII received an ip injection of naloxone (4 mg/kg) after the control period and experienced normoxemia during the experimental period (newborn n = 8; older n = 8); and experimental group VIII received an ip injection of naloxone (4 mg/kg) after the control period and experienced hypoxemia during the experimental period (newborn n = 8; older n = 8).

Condition of observations. The thermocline used in our experiments consisted of a sealed Plexiglas cylinder (2-m long, ID 0.12 m) with a plastic grid along the bottom, into which flowed room air at 1.4 l/min. A linear temperature gradient from 10 to 40°C was produced by circulating hot and cold water (Neslab, Endocal refrigerated circulating bath RTE-8DD) into two copper coils wrapped around the cylinder. Gas of the desired oxygen concentration flowed through the thermocline at a constant rate [i.e., normoxemia, 21% oxygen (room air) at 1.412 l/min; acute hypoxemia, 10% oxygen in nitrogen at 1.490 l/min]. Each time the gas mixture was changed, the thermocline was flushed by increasing the gas flow rate to ~8 l/min for 10 min.

Experimental measurements and calculations. For measurement of core temperature, platform antennas (PhysioTel CTR 86; Data Sciences International), which received the output frequency (Hz) from the previously implanted biotelemetry device, were placed under the thermocline. The received output was then fed into a peripheral processor (Dataquest III, Data Sciences International) connected to an IBM computer. Core temperature indexes for the experimental periods of normoxemia and hypoxemia were expressed as area under the core temperature curve in degrees Celsius per hour (8).

Selected ambient temperature was determined by observing the position of the guinea pig in the thermocline. Oxygen consumption was calculated by the difference between the inflow and outflow (dry) oxygen concentration (Ametek, Applied Electrochemistry S-3A/I O2 analyzer) and the flow rate.

Naloxone hydrochloride. Naloxone hydrochloride (Narcan, DuPont Pharmaceuticals) was used in doses of 1, 2, or 4 mg/kg body wt. Normal saline was used as vehicle.

Statistical analysis. Statistical analysis was carried out by using a four-factor ANOVA for repeated measures, followed by a Newman-Keuls multiple-comparison test to determine whether gas, age, drug, or time influenced core temperature, selected ambient temperature, or oxygen consumption. In addition, a four-factor ANOVA for repeated measures, followed by a Newman-Keuls multiple-comparison test, was used to determine whether gas, age, or drug influenced the core temperature index. All results are presented as means ± SD; P < 0.05 was considered to be of statistical significance.

RESULTS

With saline, core temperature decreased significantly during hypoxemia in both newborn and older guinea pigs (Figs. 1, 2, and 3). The core temperature response to hypoxemia was not significantly altered by the prior administration of any dose of naloxone, compared with the core temperature response observed after administration of saline. Neither saline nor naloxone had an effect on core temperature during normoxemia in either age group of animals.

As previously reported from our laboratory (5), selected ambient temperature decreased significantly during hypoxemia in newborn but not in older guinea pigs (Figs. 4 and 5). The decrease in selected ambient temperature, however, was not observed in the new-
born guinea pigs during hypoxemia after naloxone administration. Neither saline nor naloxone produced consistent effects on selected ambient temperature during normoxemia in either age group of animals.

With saline, oxygen consumption did not change significantly during the experimental periods of normoxemia or hypoxemia in either newborn or older animals (Figs. 6 and 7). The administration of naloxone decreased oxygen consumption during normoxemia in both newborn and older guinea pigs. This effect of naloxone on oxygen consumption, which appeared to be accentuated in newborn compared with older guinea pigs, was also observed during hypoxemia in the newborn animals at doses 2 and 4 mg/kg (Fig. 6).

**DISCUSSION**

Our data provide insight into possible mechanisms of thermoregulatory control in newborn and older guinea pigs when they are exposed to acute hypoxemia. A novel finding in our study was that naloxone did not significantly alter basal core temperature or the core temperature response to acute hypoxemia in newborn or older guinea pigs. Naloxone did, however, decrease basal oxygen consumption in newborn and older guinea pigs and altered the thermoregulatory effector mechanism used to decrease core temperature during hypoxemia in the newborn guinea pigs. Thus our data provide evidence that the endogenous opioids do not exert a tonic
influence on the central nervous system thermoregulatory set point under basal conditions in newborn and older guinea pigs. Furthermore, our data do not support the hypothesis that the endogenous opioids mediate the regulated decrease in core temperature that occurs in newborn and older guinea pigs during exposure to acute hypoxemia.

Administration of naloxone, a competitive antagonist at μ-, κ-, and δ-opioid receptors (27), did not alter basal core temperature in either the newborn or older guinea pigs. This is in agreement with the data of Kandasamy and Williams (17), who showed that an ip injection of 1–5 mg/kg of naloxone did not alter core temperature in chronically instrumented, adult male guinea pigs. Although higher doses of naloxone (e.g., 10 mg/kg sc) have been reported to produce small changes in core temperature in adult guinea pigs (2) and rats (16), it is generally accepted that the thermogenic response is too small and insensitive to warrant assigning a direct role of opioids in the maintenance of basal core temperature (6).

Administration of naloxone decreased basal oxygen consumption in newborn and older guinea pigs. This is in agreement with the data of Malin et al. (21), who showed that subcutaneous injection of 0.7 or 2.5 mg/kg of naloxone produced a decrease in oxygen consumption.
in adult rats. The mechanism of the change in oxygen consumption after naloxone is not clear. As far as we are aware, it is not known whether naloxone exerts a direct effect on brown adipose tissue activity. Egawa et al. (11) have shown, however, that subcutaneous injection of 5 mg/kg of naloxone does not affect basal sympathetic neural activity to interscapular brown adipose tissue in chloralose-urethan-anesthetized rats. Although naloxone has been reported to cause decreases in locomotor activity in adult rats, mice, guinea pigs, and rabbits (1, 10, 30, 35), we did not observe any effects of naloxone on gross motor activity in our experiments. It is possible, however, that naloxone decreased fine motor activity, which resulted in a decrease in oxygen consumption. Regardless of the mechanism, the effect of naloxone on oxygen consumption appeared to be accentuated in newborn compared with older guinea pigs. The resulting decrease in heat production after naloxone administration may have been one reason why the newborn animals did not select a cooler ambient temperature to decrease their core temperature after exposure to acute hypoxemia.

Prior administration of naloxone did not alter the decrease in core temperature that occurred in newborn
and older guinea pigs when they were exposed to acute hypoxemia. This is in contrast to the results obtained by Mayfield et al. (23) and Young and Malvin (38), who have provided evidence that endogenous opioids participate in mediating the decrease in core temperature after acute severe hypoxemia (inspired oxygen fraction 0.045) in adult mice and during acute moderate hypoxemia (inspired oxygen fraction 0.11) in adult rats, respectively. Although the reasons for the differences between their results and ours are not readily apparent, there are several possibilities.

We studied newborn and older guinea pigs that were between 5–10 and 25–30 days of age, and Mayfield et al. (23) and Young and Malvin (38) studied adult mice and rats, respectively. It seems unlikely, however, that the aforementioned differences in the effect of naloxone on the core temperature responses to acute hypoxemia in guinea pigs and rats are related to the ages of the animals at study. This is because guinea pigs (i.e., a precocial species), as opposed to some species [e.g., rats (i.e., an altricial species)], have a full complement of brain opioid receptors, as determined by stereospecific

Fig. 7. Effect of normoxemia (left) and hypoxemia (right) on oxygen consumption in older guinea pigs after an ip injection of saline or after 1 mg/kg of naloxone (A) or 2 or 4 mg/kg of naloxone (B). * P < 0.05 vs. C.
binding of \[ ^{1}H \] naltrindone in brain homogenates, in late fetal life, compared with that observed in the adult (7). Furthermore, Zhang and Moss (39) have shown that young piglets have a greater content of \( \beta \)-endorphin, Met-enkephalin, dynorphin A, and dynorphin B in several brain regions than do older piglets. Moss and Inman (25) have also shown a greater release of \( \beta \)-endorphin into the plasma and cerebrospinal fluid of young piglets during normoxemia and during hypoxemia, compared with older piglets.

It is possible that the aforementioned difference in the effect of naloxone on the core temperature responses to acute hypoxemia in guinea pigs and rats is related to a species difference in the influence of opioids on thermoregulatory control. Previous experiments carried out by Spencer et al. (31) on chronically instrumented adult rats in a thermocline have shown that intracerebroventricular administration of \([D-Ala^2, N-Me-Phe^\beta, Gly^\gamma-ol]\)-enkephalin (i.e., a specific \( \mu \)-opioid-receptor agonist) produces a regulated increase in core temperature, whereas administration of \([D-Pen^2,5]-\)enkephalin (i.e., a specific \( \delta \)-opioid-receptor agonist) and U-50,488H (i.e., a specific \( \kappa \)-opioid-receptor agonist) produces a regulated decrease in core temperature. This in contrast to the findings of Kandasamy and Williams (17), who found that intracerebroventricular administration of nonpeptide opioid-receptor agonists (\( \mu \), \( \delta \), and \( \kappa \)) as well as peptide opioids causes hyperthermia rather than hypothermia in chronically instrumented adult guinea pigs. Furthermore, Robson et al. (28) have provided evidence for species differences in the concentrations and distributions of opioid-binding sites. Thus it seems likely that the regulated decrease in core temperature during exposure to acute hypoxemia occurs via different mechanisms in rats and guinea pigs. In adult rats, it appears that the endogenous opioids participate in mediating the regulated decrease in core temperature during exposure to acute hypoxemia, whereas neither afferents from the carotid baroreceptors/chemoreceptors (14, 15, 22), an intact cerebral cortex (29), nor arginine vasopressin (4) appears to be important factors in mediating the core temperature response. In newborn and older guinea pigs, however, it appears that neither afferents from the carotid baroreceptors/chemoreceptors (13) nor the endogenous opioids play a significant role in mediating the core temperature response.

Although our experiments do not support the hypothesis that the endogenous opioids mediate the regulated decrease in core temperature during acute hypoxemia in newborn and older guinea pigs, there are a number of other possibilities to consider. It is possible that other neuromodulators, such as histamine or adenosine, which are known to increase during hypoxemia (18, 32, 36) and which are known to influence thermoregulatory control (20, 34), could mediate the regulated decrease in core temperature. Furthermore, as Tamaki and Nakayama (33) have shown that hypoxia increases the activity of warm-sensitive neurons in the preoptic area of the anterior hypothalamus, it is also possible that hypoxemia had a direct effect on these thermosensitive neurons and that this mediates the regulated decrease in core temperature. These possibilities warrant investigation.

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