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Am J Physiol Regul Integr Comp Physiol 273:R814-R822, 1997.

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McCulloch, P. F., G. P. Ollenberger, L. K. Bekar, and N. H. West. Trigeminal and chemoreceptor contributions to bradycardia during voluntary dives in rats. *Am. J. Physiol.* 273 (*Regulatory Integrative Comp. Physiol.* 42): R814–R822, 1997.—This study investigates the importance of chemoreceptive and trigeminal information during voluntarily initiated diving in rats. The heart rate responses to simulated diving are unaffected by chemoreceptor drive [McCulloch, P. F., and N. H. West. *Am. J. Physiol.* 263 (*Regulatory Integrative Comp. Physiol.* 32): R1049–R1056, 1992] but are reversibly eliminated by infusion of glutamate receptor antagonists into the spinal trigeminal nuclei [McCulloch, P. F., I. A. Paterson, and N. H. West. *Am. J. Physiol.* 269 (*Regulatory Integrative Comp. Physiol.* 38): R669–R677, 1995]. To investigate the role of chemoreceptor drive in conscious dives, rats were made hypercapnic, hyperoxic, or hypoxic predive. The role of trigeminal input was explored by infusing the glutamatergic antagonists D-2-amino-7-phosphoheptanoic acid and 6,7-dinitroquinoxaline-2,3-dione into the region of the trigeminal nuclei. The alteration of arterial blood gases predive had no effect on diving bradycardia. Trigeminal blockade reduced the intensity of the bradycardia but did not abolish it. Chemoreceptor input does not play a significant role in determining heart rate during conscious diving in rats. The attenuation, rather than abolition, of bradycardia on trigeminal blockade suggests either that we achieved incomplete blockade or that an additional spectrum of sensory inputs not present in simulated diving is important in determining the underwater heart rate during conscious diving in rats.

mammalian diving response; trigeminal pathway; chemoreceptors; spinal trigeminal nucleus interpolaris; conscious diving

ANESTHETIZED, PARALYZED rats show an intense bradycardia during simulated diving (19, 20). Lin (14) found a profound bradycardia in conscious, force-dived rats that was due to both parasympathetic activation and sympathetic withdrawal. However, there are no data on the cardiovascular responses to voluntarily initiated diving in rats. Therefore, our first objective in this study was to determine if rats show cardiovascular adjustments to voluntary diving typical of small aquatic mammals, such as muskrats, mink, and beaver (18, 27, 31).

Our second objective was to investigate the importance of chemoreceptive and trigeminal input in initiating and maintaining the cardiovascular responses to voluntary diving in rats. Preexisting chemoreceptor drive does not alter the cardiovascular responses to short simulated dives in paralyzed, anesthetized rats (20). However, blocking any part of the trigeminal neural pathway from nasal receptors to the spinal trigeminal nucleus eliminates the cardiac responses (19). The control of the cardiovascular responses to diving in conscious rats has been investigated previously, but only during forced submergence (10, 14, 15). Huang and Peng (10) came to the conclusion that the stimulation of peripheral chemoreceptors was important for the development of bradycardia during involuntary submersion, but we could not confirm this finding in anesthetized, paralyzed rats (20), in which trigeminal afferent information is of primary importance (19).

In the present study we altered chemoreceptor drive by allowing the rats to breathe hypoxic, hyperoxic, or hypercapnic gas mixtures before voluntary diving. This allowed us to investigate the role of chemoreceptor input in maintaining and initiating the cardiovascular responses to diving. Primary afferent fibers innervating the upper respiratory tract project to the spinal trigeminal nucleus (1, 21) and use glutamate as a neurotransmitter (4, 24). Therefore, we infused glutamate receptor antagonists into the region of the spinal trigeminal nucleus of voluntarily diving rats to interrupt the transfer of trigeminal neural information to other brain stem locations within the medulla (19, 22).

METHODS

The experiments reported were performed on both male and female Sprague-Dawley (n = 19) and Long-Evans (n = 5) rats (235–560 g). All procedures were approved by the Animal Care Committee of the University of Saskatchewan and were performed in accordance with the guidelines laid down by the Canadian Council on Animal Care.

Voluntary Dive Training

Rats were trained to swim underwater through a maze constructed of Plexiglas (Fig. 1). The rats were initially trained to negotiate the maze by swimming on the surface of the water. Once placed in the maze, the only exit from the water was by swimming to the finishing area. This consisted of a platform raised above the water where the animals could rest and groom between trials. The distance the rat had to swim to reach the finishing area was increased during each training session by placing Plexiglas dividers in the maze. After learning to swim through the complete maze, the rats were trained to swim underwater through the maze. Initially a sheet of Plexiglas was placed vertically, with its edge 2 cm below the surface of the water. The rat had to dive underneath this vertical Plexiglas piece to reach the finishing area. The distance the rats had to swim underwater was then progressively extended by adding horizontal subsurface Plexiglas pieces during the following training sessions. The length of the underwater dive pathway was 1.3 m, and dive duration was ~5.0 s. When placed in the starting area, rats had access to the dive pathway and voluntarily initiated their own dive to reach the rest platform, usually within 10 s. As part of the training procedure, access to the surface was temporarily blocked during a dive. This extended underwater durations to ~15–20 s. To record arterial blood pressures during diving, rats were chronically cannulated (see below). The diving tank

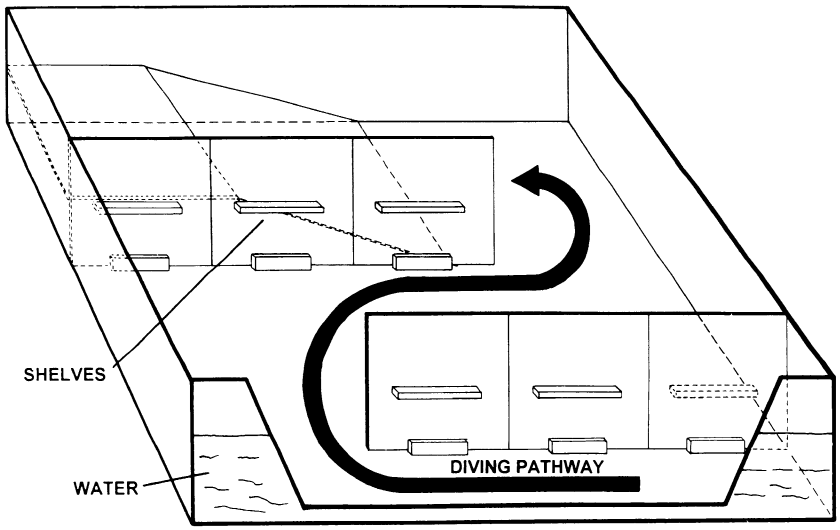

Fig. 1. Schematic drawing of dive tank that was used in recording of blood pressure and heart rate (HR) from voluntarily diving rats. Tank (70 × 45 × 25 cm) was constructed of Plexiglas. Vertical pieces inserted into tank created a simple maze consisting of 3 channels. Tank was filled with water, and rats were initially trained to negotiate maze by swimming on the surface of the water. Gradually rats learned to swim from start area, in the *lower right* of the figure to the finish area, a raised platform in the *top left* of the figure. After learning to swim through the maze, rats were trained to swim underwater through the maze. Shelves were used to hold subsurface horizontal Plexiglas pieces. Rats were trained to swim underneath horizontal pieces from start area to finish area. Underwater length of maze was ~1.3 m. A slot made in the horizontal subsurface Plexiglas pieces allowed an arterial cannula to trail along behind the rat while it swam underwater through the maze.

was designed so that the arterial cannula would trail behind the rat along a slot made in the horizontal subsurface Plexiglas pieces. The trailing cannula (PE-50; ID 0.58 mm, OD 0.965 mm; Clay Adams, Parsippany, NJ) was ~90 cm in length.

Surgical Preparation

Trained rats were anesthetized with either equithesin (3.0 ml/kg ip, diluted to a 50% solution with 0.9% saline) or Innovar-Vet (MTC Pharmaceuticals, Cambridge, Ontario; 0.15–0.2 ml/kg im, diluted to a 10% solution with saline) after initial inhalation induction with methoxyflurane (Metofane; MTC Pharmaceuticals). Buprenorphin-hydrochloride analgesic (Temgesic, Reckitt and Coleman Pharmaceuticals, Hull, UK; 0.06 mg im) was given before surgery. All surgical instruments and materials used in surgery were sterilized in an alcohol-iodine solution. The right femoral artery was cannulated with microrenethane (Braintree Scientific, Braintree, MA; ID 0.355 mm, OD 0.836 mm). The arterial cannula was filled with heparinized saline (100 IU/ml, Hepalean, Organon Teknika, Toronto, Ontario) to minimize clotting. The arterial cannula was fed subdermally to the nape of the neck, where it was attached to hypodermic tubing (23 gauge, ID 0.013 in., OD 0.025 in.; Small Parts, Miami Lakes, FL). The tubing had previously been attached to a patch of propylene screen cloth (Small Parts, 500 μm) that was implanted subcutaneously. A 1-cm tip of the metal tubing was exteriorized through the skin of the rat. The exteriorized metal tubing was attached to polyethylene tubing (PE-50; Clay Adams), which was kinked off and closed with a small piece of tubing (PE-205; ID 1.57 mm, OD 2.08 mm; Clay Adams). Incisions were tightly closed with wound clips (Autoclip, Clay Adams). The rats were left for 3–4 days to recover before dive trials started.

A detailed account of the surgical preparation involved in infusing glutamate receptor antagonists into the region of the spinal trigeminal nucleus interpolaris (Sp5I) is in Ref. 19. Briefly, at the same time as the femoral artery cannulation, two 15-mm guide cannulas (23 gauge) for infusing glutamate antagonists into Sp5I were placed bilaterally in the brain with the use of stereotaxic procedures. The coordinates used to locate Sp5I correspond to −3.80 mm posterior to interaural, ±2.8 mm lateral from midline, and 1 mm dorsal to interaural according to Ref. 23. The guide cannulas were placed 1.5 mm dorsal to Sp5I.

Experimental Procedures

Voluntary diving. Pulsatile arterial pressures were recorded from the rats as they swam underwater through the maze. The exteriorized trailing arterial cannula was attached to a pressure transducer (type 4–327-C, Beckman Instruments, Schiller Park, IL). The arterial pressure signal was connected to a cardiotachometer (Beckman type 9857B) to monitor heart rate (HR). Pulsatile arterial pressures and HR were recorded on a chart recorder writing on rectilinear coordinates (Beckman R511A). After recording control dives, some rats (n = 5) were pretreated with atropine sulfate (Sigma, St. Louis, MO; 1 mg/kg ip) to block the parasympathetic nervous system.

Alteration of chemoreceptor drive during voluntary diving. To investigate whether chemoreceptor drive alters the magnitude of the cardiovascular adjustments made during voluntary dives, rats breathed different gas mixtures before engaging in voluntary dives. This produced four groups with differing partial pressures of arterial oxygen (Pa_{O_2}) and carbon dioxide (Pa_{CO_2}). Arterial blood gases for the four groups were: *group 1*, Pa_{O_2} ~90 mmHg, Pa_{CO_2} ~25 mmHg (normoxic and normocapnic condition); *group 2*, Pa_{O_2} ~100 mmHg, Pa_{CO_2} >40 mmHg (hypercapnic condition, designed to stimulate chemoreceptors through an increased Pa_{CO_2}); *group 3*, Pa_{O_2} >200 mmHg, Pa_{CO_2} ~25 mmHg (hyperoxic condition, designed to unload peripheral chemoreceptor drive through an increased Pa_{O_2}); and *group 4*, Pa_{O_2} <60 mmHg, Pa_{CO_2} ~25 mmHg (hypoxic condition, designed to stimulate peripheral chemoreceptors through a decreased Pa_{O_2}). Inspired gases were mixed with a gas mixing pump (Digamix, model M/300 A, Wöstoff, Bochum, Germany). After breathing the gas mixture for 10 min, Pa_{O_2} and Pa_{CO_2} values were determined (IL Micro 213 pH/blood gas analyzer, Instrumentation Laboratory, Lexington, MA). Fractions of inspired N_2, O_2, and CO_2 were adjusted, as required, to ensure that blood gas values were appropriate for each group. When the blood gases were appropriate, the rats were allowed to dive underwater through the maze while pulsatile arterial pressures and HR were recorded.

Blocking the trigeminal neural pathway during voluntary diving. In an attempt to prevent afferent neural information originating from the nose and nasal passages from being integrated within the medulla, glutamate receptor antago-

nists were infused into the region of Sp5I. Infusions of glutamate antagonists were made 20 min before voluntary diving. The infusion cannulas had collars made of 23-gauge tubing, which ensured that they extended only 1.5 mm past the tips of the guide cannulas when inserted into the brain. This arrangement was designed to place the tips of the infusion cannulas within the Sp5I. Bilateral infusions were made simultaneously. The glutamate antagonists were diluted in artificial cerebrospinal fluid (CSF), and, before infusion into the brain, the solutions were warmed to 37°C in a water bath and brought to pH 7.4 by bubbling with 5% CO_2. Two glutamate receptor antagonists were used, D-2-amino-7-phosphonoheptanoic acid [AP-7; Research Biochemicals International (RBI), Natick, MA] and 6,7-dinitroquinoxaline-2,3-dione (DNQX; RBI). AP-7 is an *N*-methyl-D-aspartate (NMDA) receptor subtype antagonist, and DNQX is a non-NMDA receptor antagonist that blocks both α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate subtype receptors. To block excitatory amino acid neurotransmission at glutamatergic synapses within Sp5I, both types of receptor antagonists were used together. One microliter of equal concentrations (5 mM) of both antagonists were infused into Sp5I. Two 25-μl Hamilton microsyringes and an infusion pump (Harvard Syringe Infusion Pump 22, Ealing Scientific, St. Laurent, PQ; 1 μl/min) were used for infusions. The protocol was as follows: on the fourth day after surgery, three control dives were performed at least 20 min apart. These were followed by infusion of either Somjen's vehicle or the glutamatergic blockers (decided randomly). On the next day (*day 5* postsurgery) three control dives were performed, followed by infusion of the alternative solution. Usable data were obtained from six rats, representing 15% of the animals that started the training procedure. Forty animals were trained for this part of the protocol; however, the majority of animals did not voluntarily initiate dives after brain stem injections.

To confirm the location of infusion sites, serial histological sectioning was completed in four of six rats. At the end of the experiments, 1 μl Pontamine sky blue dye was microinjected into each guide cannula to help locate the infusion sites. The rats were injected with pentobarbital sodium (Somnotol; MTC Pharmaceuticals, 1.0 ml iv) and perfused transcardially with saline and then 4% formaldehyde. The brains were serially sectioned at 50 μm on a microtome cryostat (Minitome; Damon/IEC, Needham Heights, MA). Coronal sections of the medulla were stained with neutral red, which stains Nissl granules. The location of the infusion sites were identified with the aid of a rat stereotaxic brain atlas (23).

Statistical Analysis

Means for each animal were calculated by determining average HR and mean arterial blood pressure (MABP) from all trials. HR was calculated as the average during 2.5-s intervals. MABP was calculated from pulsatile pressure traces (diastolic plus one-third pulse pressure). There were no significant differences between the results from male and female rats or between Sprague-Dawley and Long-Evans rats. Accordingly, all data were pooled together. Grand means were calculated by averaging the means from all animals. Values reported in text and Figs. 1–7 are grand means ± SE; HR is in beats/min (bpm); and MABP is in millimeters mercury. Pre- and postdive values were calculated by averaging the responses from 0 to 5 s immediately preceding or after the voluntary dive, respectively.

Statistical analyses were performed with a computer package (SYSTAT, Systat, Evanston, IL). One- and two-way analyses of variance with repeated measures were employed, as required (13, 32). Significance was reached when $P < 0.05$. In the case of significant F values, Tukey's honestly significant difference a posteriori tests were performed to determine significant differences among group means.

RESULTS

Rats were easily trained to dive and swim through the maze. The full training procedure was completed within a few weeks. After initial orientation to the water the rats appeared to be quite calm and unconcerned about entry into the water. About one-half the rats got a slightly bloody nose while diving, seemingly due to water entry into the nasal passages. This did not interfere with their diving behavior or change their cardiovascular responses to diving.

Cardiovascular Responses During Voluntary Diving

Voluntary diving in rats resulted in an immediate bradycardia (Fig. 2). HR decreased by 82.6% (478 ± 13 to 83 ± 16 bpm) within a single beat and remained at this level for the duration of the dive (Fig. 3*A*). MABP initially decreased slightly (Fig. 2), but then increased and remained at greater than predive values for the duration of the dive (Fig. 3*B*). MABP increased by a maximum 18.5% (137 ± 5 to 162 ± 11 mmHg) at 7.5 s.

Pretreatment with atropine eliminated the bradycardia associated with voluntary diving (Fig. 3). HR did not change significantly during the dive (Fig. 3*A*). Atropine significantly increased predive HR by 19.5%, compared with the nonatropine condition (Fig. 3*A*). Pretreatment with atropine caused a substantial increase in MABP during voluntary diving (Fig. 3*B*). MABP increased from 130 ± 6 to 202 ± 5 mmHg at 7.5 s.

Effects of Changing Chemoreceptor Drive

Rats were ventilated with different gases to change chemoreceptor drive. Pa_{O_2} and Pa_{CO_2} for the four groups are presented in Table 1. Altering the Pa_{O_2} or Pa_{CO_2} did not have any effect on the cardiovascular responses to voluntary diving (Fig. 4). Not all dives from all animals were of the same duration nor were there the

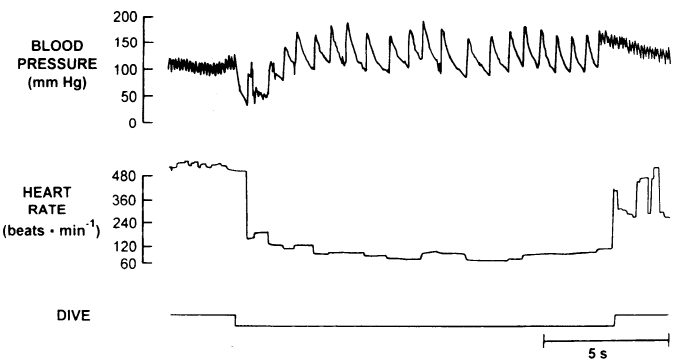

Fig. 2. Original recording of pulsatile arterial pressure and HR from a voluntarily diving conscious rat trained to swim through underwater maze. Submersion produced an immediate bradycardia and hypotension. Although the bradycardia was sustained, a few seconds after submersion hypotension was eliminated and arterial blood pressure rose above predive levels. Dive is indicated by downward movement of event marker.

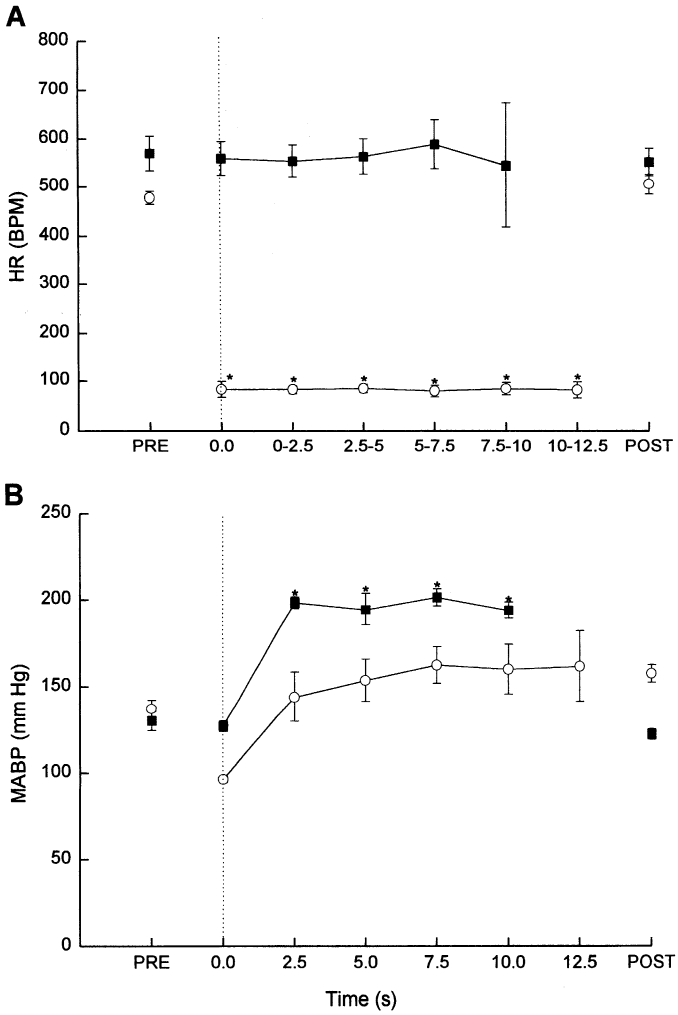

Fig. 3. HR (*A*) and mean arterial blood pressure (*B*; MABP) during voluntary diving in 5 rats before (○) and after (■) atropine pretreatment. Symbols represent mean values ± SE. Before atropine pretreatment there was an 82.6% decrease in HR and a maximum 18.5% increase in MABP during voluntary diving. Atropine eliminated the bradycardia associated with diving and caused a 55.5% increase in MABP. Vertical dotted line indicates beginning of dive. Pre and Post represent predive and postdive values, respectively. Not all dives from all animals lasted 12.5 s. Before pretreatment with atropine, $n = 5$ at Pre, 0.0, 2.5, 5.0, and Post; $n = 4$ at 7.5; $n = 3$ at 10.0; $n = 2$ at 12.5. After pretreatment with atropine, $n = 5$ at Pre, 0.0, 2.5, 5.0, and Post; $n = 3$ at 7.5; and $n = 2$ at 10.0. Atropine had a significant treatment effect on both HR and MABP. *Value is significantly different from its predive value at $p < 0.05$. bpm, Beats/min.

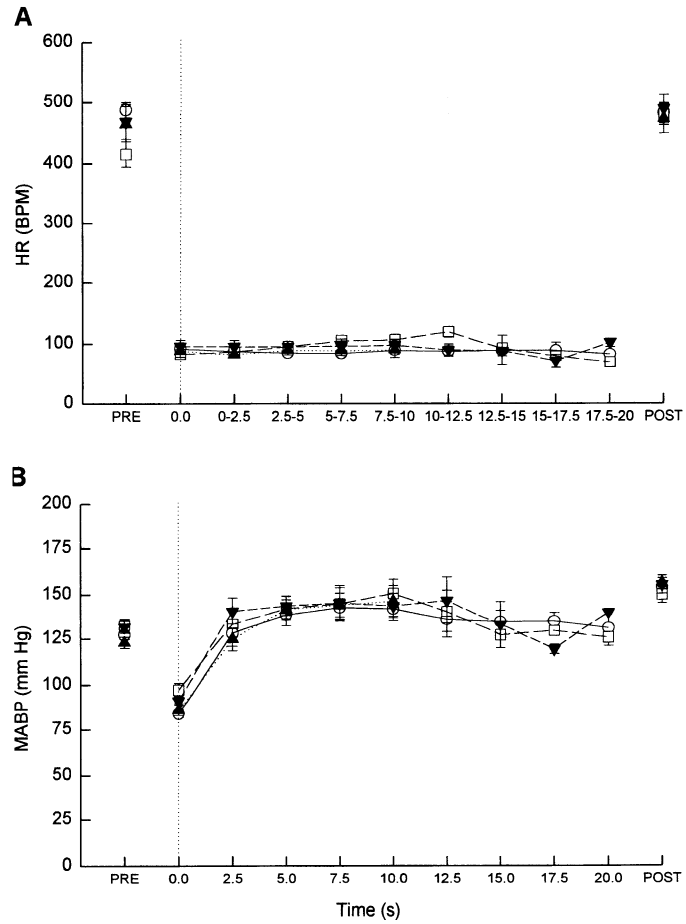

Fig. 4. HR (*A*) and MABP (*B*) responses during voluntary diving after changing preexisting chemoreceptor drive. Breathing different gases before voluntary diving altered arterial P_{O_2} and P_{CO_2} (see Table 1). *Group 1*, control-normoxic and normocapnic condition (○); *group 2*, hypercapnic condition (□); *group 3*, hyperoxic condition (▼); and *group 4*, hypoxic condition (▲). Symbols represent mean values ± SE. In all 4 groups HR decreased from ~450 to 90 bpm immediately on submersion. HR remained at this level for the rest of the dive. MABP decreased from ~130 to 90 mmHg immediately on submersion. MABP then increased to a peak of ~145 mmHg around 10.0 s, but then slowly decreased to near predive levels by 20.0 s. Vertical dotted line indicates beginning of dive. Pre and Post represent predive and postdive values, respectively. Not all dives from all animals lasted for the same duration (see Table 2). Altering arterial P_{O_2} and P_{CO_2} did not have significant treatment effects on either HR or MABP for any of the 4 groups.

same number of animals in each group and none in the hypoxic group after 10 s (see Table 2).

In the normoxic and normocapnic condition (*group 1*), HR decreased from 488 ± 12 to 92 ± 8 bpm immediately on submersion (Fig. 4). HR remained at this level for the remainder of the dive. MABP decreased from 127 ± 3 to 84 ± 2 mmHg immediately on submersion (Fig. 4). MABP increased to a peak of 143 ± 8 mmHg at 7.5 s, but then slowly decreased to near predive levels by 20.0 s.

Preexisting chemoreceptor drive was increased before submersion by increasing Pa_{CO_2} (*group 2*, hypercapnic condition) or by decreasing Pa_{O_2} (*group 4*, hypoxic condition). There were no differences in the HR

Table 1. *Pa_{O_2} and Pa_{CO_2} values for 4 groups ventilated with different gases before voluntary diving*

Group	Pa_{O_2}	Pa_{CO_2}
1	89.9 ± 2.0	26.3 ± 1.3
2	106.5 ± 1.9	39.9 ± 0.7‡
3	255.2 ± 16.0*	26.2 ± 1.4
4	54.0 ± 2.5†	22.1 ± 0.7

Values are means ± SE in mmHg ($n = 8$ for *groups 1, 2,* and *3*; $n = 6$ for *group 4*). *Group 1,* normoxic-normocapnic; *Group 2,* hypercapnic; *Group 3,* hyperoxic; and *Group 4,* hypoxic. *Significantly greater than all other arterial P_{O_2} (Pa_{O_2}) values; †significantly less than all other Pa_{O_2} values; ‡significantly greater than all other arterial P_{CO_2} (Pa_{CO_2}) values. Values are significant at $p < 0.05$.

Table 2. *n Numbers used in calculating HR and MABP for 4 groups ventilated with different gases*

Group	Pre	0.0	2.5	5.0	7.5	10.0	12.5	15.0	17.5	20.0	Post
1	13	13	13	13	11	10	9	7	7	4	13
2	11	11	11	11	6	5	2	1	1	1	11
3	9	9	9	9	6	6	4	2	2	1	9
4	9	9	9	9	7	4					9

Dive durations (in s) were not the same for all animals nor for all groups. Pre and Post represent predive and postdive, respectively. HR, heart rate; MABP, mean arterial blood pressure.

or MABP responses between either of these two groups and *group 1* (Fig. 4), although the hypoxic group had reduced dive durations (Table 2). Preexisting peripheral chemoreceptor drive was unloaded by increasing Pa_{O_2} (*group 3*, hyperoxic condition). There was no difference between the HR and MABP for *groups 3* and *1* (Fig. 4).

Because there were no significant differences between chemoreceptor treatment groups, HR data were pooled to investigate the relationship between predive and dive HR. Higher predive HRs resulted in slightly higher dive HRs recorded at 5, 10, and 15 s (regression equation, $y = 29.4 + 0.13x$).

Effects of Infusing Glutamate Receptor Antagonists Into Sp5I

Histological examination of the brains from four of six rats used for this experiment confirmed that glutamate receptor antagonists and/or artificial CSF were bilaterally injected into the region of and surrounding the dorsal trigeminal nucleus (Fig. 5, *A-C*). The brain of one animal showed incomplete fixation, whereas one brain was sectioned too far rostrally to show the infusion sites; consequently, these brains were not used for histological analysis.

Without any infusions into the region of Sp5I or with infusion of artificial CSF only, voluntarily diving rats displayed a marked bradycardia and MABP was maintained slightly above resting values (Fig. 6*A*). There were no statistical differences between these two conditions. After infusion of artificial CSF into the region of Sp5I, HR decreased 75% (499 ± 18 to 124 ± 16 bpm) within 2.5 s after submersion and was maintained at this level for the duration of the dive (Fig. 7*A*). MABP

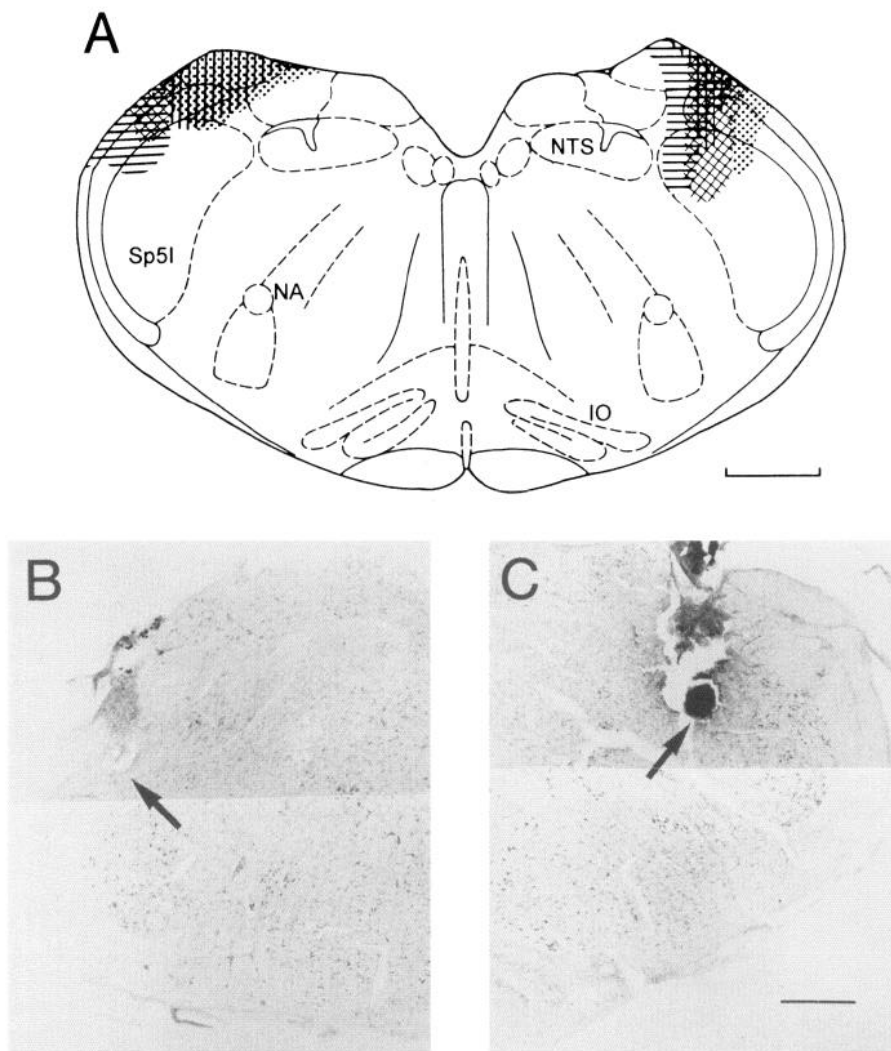

Fig. 5. Coronal cross section of the medulla indicating location of injection sites when glutamate receptor antagonists were infused into the spinal trigeminal nucleus interpolaris (Sp5I). *A*: line drawing with crosshatched or stippled areas showing extent of tissue damage caused by cannula track in each of 4 rats. Ventral apex of each area represents site of the cannula tip. Histological examination confirmed that glutamate receptor antagonists and/or artificial cerebrospinal fluid (CSF) were bilaterally infused into the region of and surrounding Sp5I. Bar, 1 mm. NTS, nucleus of the solitary tract; NA, nucleus ambiguus; IO, inferior olive. Section is 3.80 mm posterior to interaural (from Ref. 23). *B* and *C*: photomicrographs of left and right cannula tracks and infusion sites in 1 rat. Arrows point to the ventral location of infusion sites. Bar in *C*, 500 μm for both *B* and *C*.

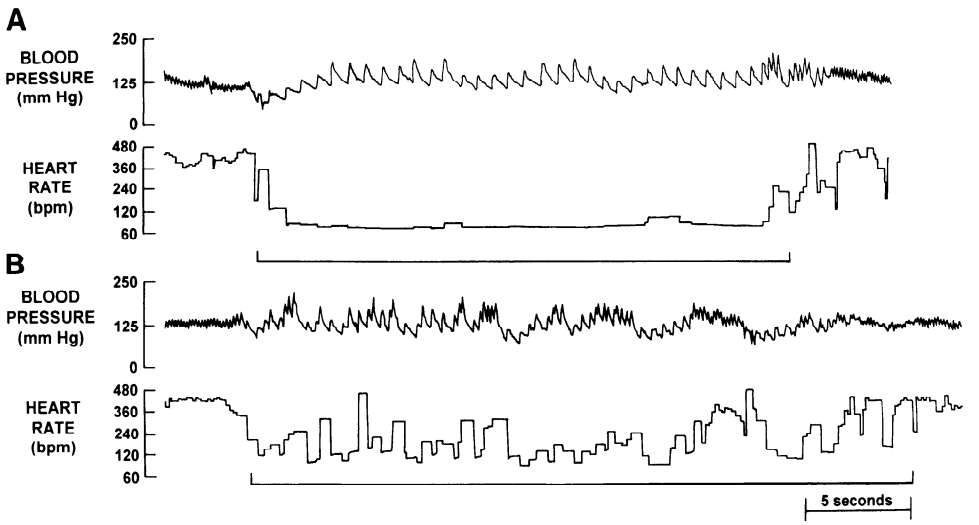

Fig. 6. Recordings of pulsatile arterial pressure and HR from a voluntarily diving rat after bilateral infusion of glutamate receptor antagonists into Sp5I. *A*: after infusion of 1 μl vehicle (artificial CSF with no antagonists); *B*: after infusion of 1 μl glutamate receptor antagonists D-2-amino-7-phosphoheptanoic acid (AP-7) and 6,7-dinitroquinoxaline-2,3-dione (DNQX; 5 mM each). Infusion of glutamate receptor antagonists changed the cardiovascular responses to voluntary diving. After infusion of the antagonists, the resultant bradycardia was not as intense nor was it sustained throughout the dive. Also, the initial hypotension at the beginning of the dive was eliminated after antagonist infusion. Dives are indicated by the downward movement of the event marker.

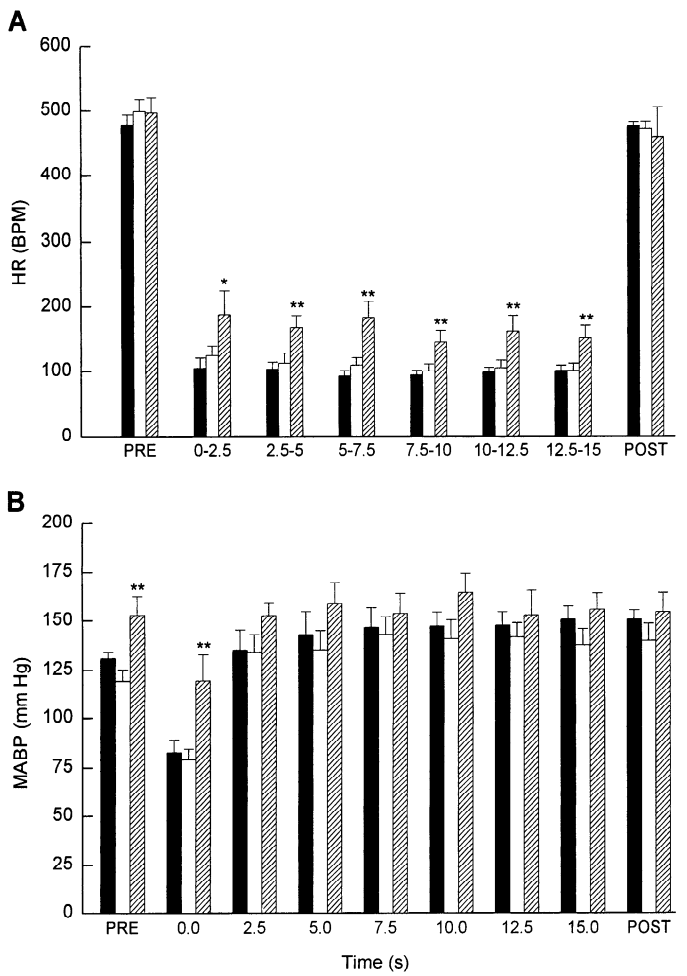

Fig. 7. HR (*A*) and MABP (*B*) responses ± SE during voluntary diving before and after 1 μl of glutamate receptor antagonists AP-7 and DNQX (5 mM each) were infused into Sp5I. No infusions, solid bars; after artificial CSF only, open bars; and after antagonist infusion, hatched bars; $n = 6$. Infusion of glutamate receptor antagonists significantly attenuated the bradycardia responses to voluntary diving, but MABP was not significantly different from controls. *Significantly different from no-infusion condition at that time; **significantly different from both other values at that time.

decreased 34% immediately on submersion (119 ± 6 to 79 ± 5 mmHg), but increased to 13% above predive (135 ± 10 mmHg) by 5 s into the dive (Fig. 7*B*).

Infusion of glutamate receptor antagonists into the region of Sp5I significantly changed the cardiac response to voluntary diving (Fig. 6*B*). Although control HRs were identical, diving bradycardia was not as intense after antagonist infusion. Within 2.5 s after submersion, HR had decreased only 62% (497 ± 22 to 188 ± 36 bpm) and remained near this level throughout the duration of the dive (Fig. 7*A*). Diving bradycardia was significantly attenuated after infusion of glutamate receptor antagonists compared with either no infusion or infusion of artificial CSF. After infusion of antagonists into the region of Sp5I, MABP decreased by 22% (152 ± 10 to 119 ± 13 mmHg) immediately on submersion but quickly increased to predive (153 ± 7 mmHg) at 2.5 s (Fig. 7*B*). MABP was not significantly different from control pressure, except at predive and immediately on submersion, when it was significantly higher.

DISCUSSION

Cardiovascular Responses to Voluntary Diving in Rats

Voluntary diving resulted in an immediate and sustained bradycardia that was eliminated after pretreatment with atropine. Therefore, in our experiments the bradycardia was entirely of parasympathetic origin. Lin (14) found that diving bradycardia in conscious rats was due to both parasympathetic activation and sympathetic withdrawal. However, these were forced-dive rats, which almost certainly had an increased sympathetic tone due to stress. Presumably the voluntary diving protocol eliminated the stress component associated with forced submergence (7). MABP during voluntary diving was maintained at or slightly above predive values despite the bradycardia, suggesting that there was significant peripheral vasoconstriction. Arterial pressures are also

maintained at or above predive levels in conscious rats during forced submergence (10, 14, 15).

The cardiovascular responses of voluntarily diving rats are similar, both qualitatively and quantitatively, to the responses in other small mammals, such as muskrats (6, 12, 16, 18), mink (31), and beavers (27). It is not surprising that rats exhibit the typical mammalian cardiovascular diving response (2, 3, 5). Feral *Rattus norvegicus* are excellent swimmers and are often found along ditches, rivers, and marshes (9, 11, 29). Rats have an innate ability to swim (25), and, as we found, can be easily trained to swim underwater.

Afferent Basis of the Cardiovascular Responses to Voluntary Diving in Rats

Role of chemoreceptor drive during voluntary diving. Chemoreceptor drive is not an important factor in the initiation or maintenance of the cardiovascular responses to voluntary diving in rats, at least over the dive times we recorded. Increasing or decreasing preexisting chemoreceptor drive before submersion did not significantly change HR or MABP responses to voluntary diving (Fig. 4). The findings from the present study are contrary to those reported by Huang and Peng (10), who concluded that peripheral chemoreceptors play an important role in diving in conscious forced-dive rats. However, they are consistent with the results of McCulloch and West (20), who found that preexisting chemoreceptor drive did not influence the cardiovascular responses to simulated diving (nasal water flow plus apnea) in rats. Our results are in accord with Daly's (5) statement that carotid body chemoreceptors do not contribute to the initiation of diving bradycardia in mammals but may maintain or increase the bradycardia toward the end of long-duration dives.

It is difficult to assess the role of another group of receptors potentially involved in the cardiovascular responses, the arterial baroreceptors. In the diving rat, there is little change in mean arterial pressure despite increased vascular tone and decreased cardiac output, suggesting that any resetting of the baroreflex is secondary to the stimulation of trigeminal receptors and the cessation of ventilation, which results in progressively increasing chemoreceptor input (5). Indeed, it has been shown in birds that the arterial baroreflex is progressively attenuated as the result of an occlusive central interaction with the increasing input from arterial chemoreceptors during the course of the dive (26).

Role of the trigeminal neural pathway. We attempted to block the trigeminal pathway centrally in voluntarily diving rats by infusing glutamate receptor antagonists into the region of Sp5I. The amino acid L-glutamate functions as an excitatory neurotransmitter within the trigeminal system (4, 17, 30). It also appears to mediate synaptic transmission at the central terminals of primary afferent neurons (4, 24). Infusion of glutamate receptor antagonists into Sp5I reversibly eliminated the cardiac response to simulated diving (nasal water flow plus apnea) in rats (19). We anticipated that infusion of glutamate receptor antagonists into the region of Sp5I might have the same effect in voluntarily diving rats. This was not the case. Bradycardia was significantly attenuated throughout the dive, but not eliminated.

There are two possible explanations as to why the results we obtained differed from those found in simulated diving although the infusion protocol was the same.

1) We may not have achieved complete blockade of glutamatergic synaptic transmission in Sp5I in the current experiments. Histology confirmed that glutamate receptor antagonists were infused into the region of and surrounding Sp5I (Fig. 5). The major focus of the injection sites was the dorsal portion of Sp5I, although in some injections the ventral aspect of the injection sites barely reached the dorsal border of the nucleus (Fig. 5*A*, *left*). We infused a large volume of antagonists. The same infusion volume reversibly eliminated the cardiac responses in rats undergoing simulated diving (19), suggesting that the abolition of bradycardia was not due to local pressure or distortion of central nervous system anatomy by the infusate. However, the possibility exists in the current experiments that either nuclei outside Sp5I participated in the voluntary responses or the results reflect unilateral rather than bilateral blockade of trigeminal transmission during voluntary dives.

In the simulated diving preparation, only the receptive fields of the ethmoidal nerve and the posterior nasal nerve, both of which innervate the nasal passages, were stimulated by retrograde flow of water. These receptive fields would not be stimulated so intensely in conscious diving, even if water entered the nose. On the other hand, in conscious diving rats the external nares and skin of the nose, face, lip, and eyelid, innervated by nerves from both ophthalmic and maxillary divisions of V, would be stimulated by the surrounding water. Therefore, the pattern of synaptic activity within the trigeminal nucleus would almost certainly differ in the two protocols, although in both cases the stimulus was adequate to elicit a rapid and intense bradycardia. Horseradish peroxidase neuronal tracer studies indicate that the trigeminal nerves innervating the nasal passages project to diverse locations within the relatively large trigeminal nucleus (21, 28). Panneton and Yavari (22) have recently shown in muskrats that nanoliter injections of glutamate antagonists into the ventral trigeminal subnucleus caudalis block the responses to ammonia stimulation of the nasal passages. This suggests a particularly high density of nasal trigeminal primary afferent projections to this part of the nucleus.

2) Although the trigeminal afferent inputs may have been eliminated in both conditions by infusion of glutamate receptor antagonists into Sp5I, consciously diving animals experience an additional spectrum of sensory inputs not present in simulated diving. These include suprabulbar inputs, including descending cortical input (18), proprioceptive feedback from exercising muscles, and water pressure and/or temperature sensed at other sites. These additional inputs may have contributed to the determination of intensity of bradycardia

during voluntary diving after antagonist infusion. Thus, during voluntary diving, the bradycardia after antagonist infusion into the region of Sp5I was attenuated rather than eliminated. A closely comparable result to ours was obtained in diving ducks by Furilla and Jones (8). They found that abolition of nasal receptor input by local anesthesia removed only 10–30% of the initial HR reduction in voluntary dives in the redhead duck (*Aythya americana*) compared with 80% in forced dives.

We did not rule out a third possibility that blockade of a site outside of the spinal trigeminal nucleus, such as the nucleus of the solitary tract (NTS), was responsible for the attenuation of diving bradycardia in these experiments. In anesthetized, paralyzed rats, similar glutamatergic blockade of the trigeminal nucleus abolished bradycardia in animals with an intact baroreflex (19), suggesting that the cardiovascular effect was not due to glutamatergic synaptic blockade at the NTS.

Our data, therefore, suggest that information transmitted by trigeminal afferents is significant in both the initiation and maintenance of bradycardia in voluntarily diving rats. Central synaptic blockade of the trigeminal neural pathway significantly attenuated, but did not abolish, bradycardia during the entire dive. This is in contrast to the results we obtained in a simulated diving preparation (19), in which bradycardia was abolished. As stated above, it is possible that we achieved only incomplete blockade of the trigeminal input in the current experiments. However, other afferent inputs that are not present in simulated diving, such as suprabulbar inputs and input arising from the exercising limbs, may also be involved in determining the intensity of bradycardia in conscious diving. We have, however, ruled out one such input, chemoreceptor activation, as an important factor in initiating or maintaining bradycardia in short, conscious dives in rats.

Perspectives

Although much attention has been focused on the receptors and afferent neural pathways that mediate the cardiovascular responses to diving in mammals and birds (2, 3), most information has been derived from experiments in which the cardiovascular effector output is changed by receptor denervation or stimulation during simulated diving. Almost no attempts have been made to investigate the central neural integration of such information, although this represents a logical investigative step. Furthermore, the role of the receptors in conscious diving, in which there is cerebral involvement (18), is far from clear.

In this paper we attempted to interfere with the central synaptic transmission of trigeminal sensory information important for the cardiovascular responses to simulated diving in the rat (19), which shows a conscious diving response typical of small semiaquatic mammals. No previous attempts have been made to determine the cardiovascular effects of central synaptic blockade of a potentially important afferent pathway during conscious diving. Further refinements to this approach hold promise for a better understanding of the role of receptor input and its central integration in the mammalian diving response.

This work was funded by a Natural Sciences and Engineering Research Council research grant to N. H. West. P. F. McCulloch was supported by a Heart and Stroke Foundation of Saskatchewan scholarship and by University of Saskatchewan graduate scholarships. G. P. Ollenberger is the recipient of a Research Traineeship from Heart and Stroke Foundation of Canada.

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Received 25 April 1996; accepted in final form 10 April 1997.

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