Autonomic nervous control of cardiovascular response during diving in the rat

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LIN, Y. C. Autonomic nervous control of cardiovascular response during diving in the rat. Am. J. Physiol. 227(3): 601–605. 1974.—The diving response of the unanesthetized rats was produced by submerging the head up to eye level in 30°C water by head-down tilting. The head-down tilting without head immersion served as the predive control. A steady response of blood pressure (BP) and heart rate (HR) to diving was established within 5 s following head immersion. HR was reduced by 64% and BP increased by 15% during diving. Sympathetic tone was reduced by 61% and parasympathetic tone increased 306% from the predive values, whereas intrinsic heart rate was not altered during diving, as deduced from selective autonomic blockade by atropine or propranolol. Cardiac output was reduced by 79% and total peripheral resistance increased fourfold from the predive values. Stroke volume was not changed, stroke work increased by 24%, and left ventricular power decreased by 65% during diving. The unanesthetized rats indicated a diving response qualitatively and quantitatively similar to that of diving mammals rather than nondiving species. It is a potential model for diving studies so far as cardiovascular responses are concerned.

Cardiac output; aortic blood pressure; heart rate; total peripheral resistance; cardiac power; apnea

APPROPRIATE HEMODYNAMIC ADJUSTMENTS enable the diving mammals to effectively channel the limited amount of oxygen preferentially to the heart and the brain (1, 31). The work requirement of the heart is lowered during diving in diving mammals, by instantaneous and intense bradycardia, and reduction in cardiac output, while central systemic arterial blood pressure is maintained at normal level, reflecting severe peripheral vasoconstriction (4, 21). On the contrary, nondiving species (nonnaturally diving species), such as man and dog, develop bradycardia gradually and to a much less extent (4, 11, 15, 30), change in cardiac output is negligible during breath-holding in man (11, 30), and hypertension develops gradually but continuously during the course of breath-holding in man (11) and in the dog (19). The present study is aimed at comparing the diving responses of a nondiving species, the rat, to that of the classical description of a diving mammal and to that of man.

METHODS

Preparation of animal. Male Wistar white rats weighing 350–405 g were surgically prepared, under pentobarbital (30 mg/kg) anesthesia, as follows: an aortic catheter was introduced via the left carotid artery for purposes of recording blood pressure and collection of blood samples for cardiac output determination. A right-atrial catheter was introduced via the right jugular vein for purposes of injection of indicator during cardiac-output determinations. These catheters were threaded subcutaneously, with their exits at the back of the head between the two ears, sutured onto the skin. The animals were allowed to recover for a minimum of 3 days prior to experimentation.

Diving response. The diving response of the unanesthetized rats was produced by submerging the head up to the level of the eyes in 30°C water by head-down tilting. The surgically prepared rat (see above) was confined in a wire-mesh cone for the diving maneuver. The head-down tilting position without head immersion served as predive control.

Systemic arterial blood pressure. This pressure was recorded with a Statham P23Db transducer and a Beckman Dymograph, via the implanted aortic catheter. The transducer was mounted at the level of the heart, and it was arranged so that the level of the transducer changed in accordance with the level of the heart during the diving maneuver. The mean arterial blood pressure was determined planimetrically over a 5-s steady-state response during diving.

Heart rate. Heart rate (HR) was counted from the pressure tracings. It was confirmed on several occasions that the pressure wave can be used to assess the heart rate during the diving response by recording the electrocardiogram and blood pressure simultaneously.

Autonomic control of heart rate. Autonomic control was deduced from selective blockade of sympathetic or parasympathetic nervous activity by treating the rat with propranolol hydrochloride (8 mg/kg) or atropine sulfate (1 mg/kg) according to a procedure described by Lin and Horvath (18). We could have administered these drugs via the implanted right atrial catheter, but we administered them intraperitoneally since a procedure, using intraperitoneal injection, had been established previously regarding dosage and time course of the effectiveness of the drugs (18).

Heart rates obtained at 20 min after the administration of atropine and/or propranolol were used in the above computations since the sympathetic blockade by propranolol was effective for a prolonged time, but the response to atropine began to decline 20 min after injection. The 20-min time interval, therefore, represented the most effective blockade in both divisions of the autonomic nervous system, and because of this the diving experiments were performed 20 min after the injection of blocking drugs.

Reserpine treatment. The importance of peripheral catechol-
amine stores on the integrity of the cardiovascular response to diving was examined by pretreating the animal with reserpine sulfate (5 mg/kg). The diving response was initiated before treatment and 4 h posttreatment with reserpine.

Cardiac output. Output was calculated from the dilution data using cesium-137 as an indicator, taking advantage of its long half-life (30 yr), gamma-emitting property. The indicator (10 μCi/kg), in a volume of less than 0.5 ml, was injected into the right atrium via the implanted right atrial catheter and serial arterial blood samples were collected via the implanted aortic catheter onto a motorized collecting disk. The circular disk was equipped with 60 depressions located on the outer perimeter and spaced 1 s apart. The motor (Universal timer, Dimco-Gray Company, Dayton, Ohio) rotated at precisely 1.0 rpm. Ten microliters of blood were measured from each collected depression using disposable pipettes (Eppendorf Brinkmann Instruments, Westbury, N. Y.) and the radioactivity of each sample was counted in a Packard gamma counter. The downslope of the dilution curve was reconstructed to exclude the recirculation of the indicator. Both the reconstruction of dilution curve and the calculation of the cardiac output were performed in accordance with the method of Kinsman et al. (16).

Cardiac outputs were determined after heart rate and aortic blood pressure were recorded, once before and once during diving, in the same rat. The blood radioactivity level increased following the first injection of the indicator and this is subtracted from each sample for the second determination. Cardiac outputs, predive and during dive, were determined randomly. When cardiac output during dive was determined first, 0.5 h was allowed for resting immediately after the dive.

RESULTS

Control response to diving. A typical response to diving as initiated by immersion of the head of a rat in 30°C water is shown in panel A of Fig. 1. Initiation and termination of a dive are indicated by left and right arrows, respectively. The 1-s mark is indicated between panels B and C. Arterial blood pressure increased immediately (1-2 s) following head immersion, and a prominent bradycardia developed 1-3 s later. A steady response of arterial blood pressure and heart rate to diving was established within 5 s following the head immersion. Blood pressure and heart rate values were averaged over the 10-s period after the steady response was obtained.

Heart rate and mean arterial blood pressure responses. These responses to head immersion of the normal (control, saline injected), atropine-, propranolol-, or reserpine-treated rats are presented in Fig. 1 and in Table 1.

The control rats responded to diving by a 64% reduction (P < 0.001) in heart rate and 15% increase (P < 0.01) in mean arterial blood pressure. Atropine treatment did not abolish the bradycardia response completely, but the magnitude of reduction in heart rate was much less than that of control rats. Heart rate was reduced by 10% (P < 0.05) from the predive value. Mean arterial blood pressure increased by 37% (P < 0.001) from the predive value. Propranolol treatment decreased the predive heart rate from 399 ± 12 to 297 ± 9 (P < 0.001). A 65% reduction (P < 0.001) in heart rate from the predive value was observed during diving in the propranolol-treated rats. The magnitude of this reduction was similar to that of the control group. For reasons unclear to us, the arterial blood pressure was more variable following propranolol treatment than that following atropine or reserpine (Table 1). Mean arterial blood pressure rose 21% above the predive value (0.1 > P > 0.05). Reserpine treatment depressed predive values of both heart rate and mean arterial blood pressure as compared to the control rats. Diving bradycardia was still present but the magnitude of the bradycardia was reduced when compared to the control group. Hypotension, rather than hypertension, was observed during diving in reserpine-treated rats, signifying failure of peripheral vasoconstriction in the face of cardiac output reduction (Fig. 1).

Autonomic nervous control of heart rate. Autonomic control of heart rate was deduced from selective blockade of sympathetic or parasympathetic nervous activity. The primary results, obtained from Table 1, along with the calculated results are shown in Table 2. The heart rate free of autonomic control—the intrinsic heart rate HR−—was essentially unchanged (+4%). Sympathetic tone was reduced 61%
HEMODYNAMICS DURING DIVING

TABLE 2. Autonomic nervous control on heart rate of unanesthetized rats before and during diving

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Predive</th>
<th>Dive</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>399 ± 12</td>
<td>143 ± 6</td>
<td>-64</td>
</tr>
<tr>
<td>Atropine, 1 mg/kg*</td>
<td>404 ± 8</td>
<td>418 ± 17</td>
<td>-10</td>
</tr>
<tr>
<td>Propranolol, 8 mg/kg*</td>
<td>297 ± 9</td>
<td>103 ± 23</td>
<td>-65</td>
</tr>
<tr>
<td>Intrinsic heart rate, HR&lt;sub&gt;s&lt;/sub&gt;†</td>
<td>302</td>
<td>370</td>
<td>+4</td>
</tr>
<tr>
<td>Sympathetic tone, % HR&lt;sub&gt;s&lt;/sub&gt;‡</td>
<td>28.2</td>
<td>10.6</td>
<td>-61</td>
</tr>
<tr>
<td>Parasympathetic tone, % HR&lt;sub&gt;s&lt;/sub&gt;‡</td>
<td>17.9</td>
<td>72.6</td>
<td>+306</td>
</tr>
</tbody>
</table>

Values are means ± SE (six rats in each experiment). *The dive was performed 20 min after the administration of drug(s). †Calculated from selective blockade of the divisions of autonomic nervous system by atropine or propranolol. See text. ‡Sympathetic tone and parasympathetic tone represent the percentage of intrinsic heart rate that is controlled by sympathetic activity and by parasympathetic activity, respectively. They are expressed as percentages of the intrinsic heart rate. See text.

TABLE 3. Hemodynamic changes in unanesthetized rats before and during diving

<table>
<thead>
<tr>
<th></th>
<th>Predive</th>
<th>Dive</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>401±17</td>
<td>114±16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean systemic arterial blood pressure, mmHg</td>
<td>111±10</td>
<td>144±10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>91±10</td>
<td>27±6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stroke volume, ml/beat</td>
<td>0.227±0.040</td>
<td>0.237±0.059</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Stroke work, g m/beat</td>
<td>0.34±0.5</td>
<td>0.47±0.13</td>
<td>&gt;0.3</td>
</tr>
<tr>
<td>Left ventricular power, g-m/min</td>
<td>137±22</td>
<td>53±12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total peripheral resistance, (dynes-cm&lt;sup&gt;-1&lt;/sup&gt;)·10&lt;sup&gt;–2&lt;/sup&gt;</td>
<td>0.973±0.137</td>
<td>4.256±1.08</td>
<td>&lt;0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE, six rats in each group.

and parasympathetic tone increased 306% from the predive values during diving.

Cardiac output. Cardiac output obtained from a separate group of six rats during diving indicated a 70% reduction (P < 0.01) from the predive value (Table 3).

Hemodynamic profile. Hemodynamic changes during diving are given in Table 3. Stroke volume, stroke work, left ventricular power, and total peripheral resistance were calculated from the measured parameters. Stroke volume was not changed (P > 0.9), and stroke work was also unchanged (P > 0.3); left ventricular power decreased by 61% (P < 0.01), whereas total peripheral resistance increased fourfold (P < 0.02).

DISCUSSION

Since the discovery of diving (apneic) bradycardia in the duck just over 100 yr ago (3), a similar response has been demonstrated in many other vertebrates including man (1, 25, 27). Moreover, it has been shown that fish taken out of water (diving in reverse) exhibit profound bradycardia (9, 17), indicating the universality of the bradycardia response to respiratory arrest, differing among species only in the magnitude of the response. It is in general agreement that the final common pathway of the diving-bradycardia response is the vagus nerve in all species thus far reported, as ascertained by vagotomy or by atropine administration. Our observation, a threefold increase in parasympathetic tone (Table 2), is in consonance with this view. The contribution of sympathetic nervous activity to the bradycardia response, however, has not been reported previously. Our results indicate a 61% reduction in sympathetic tone during diving, in the face of a 306% increment in parasympathetic tone from predive values, as far as heart rate control is concerned. Thus, the diving bradycardia in the rat is produced by concomitant elevation of parasympathetic tone and reduction in sympathetic tone, with parasympathetic control dominating.

The circulatory response to diving consists of various intensities of bradycardia and peripheral vasoconstriction with or without alterations in the mean arterial blood pressure among mammals with wide ranges of tolerance to respiratory arrest. The rat exhibits greater and faster developing bradycardia than that of man, but they are similar in hypertensive response. The central systemic arterial blood pressure is maintained at the predive level in marine mammals (11, 20, 21), nutria (6), and beaver (12, 25). The diving hypertensive response observed in the unanesthetized rat in our study, on the other hand, is consistent with the observations in man (11), in the dog (4, 19), in the cat (5), and in the sheep (28).

The dosages of atropine and propranolol we used in this study represent our own lengthy trials of lowest dosage for the highest heart rate attainable with atropine sulfate (1 mg/kg) at resting condition and also for the lowest heart rate attainable with propranolol hydrochloride (8 mg/kg). The dosages that we tried are 0.5, 1, 2, and 5 mg/kg, and 1, 2, 4, 8, and 16 mg/kg for atropine and propranolol, respectively. The reasons for expressing autonomic tone in terms of the fractions of intrinsic heart rate rather than the recorded heart rate have been discussed previously (18). Under conditions in which HR and HR<sub>s</sub> vary in the same direction, such as during exercise, the use of HR as the denominator will result in underestimation of sympathetic tone, where HR<sub>s</sub> is the heart rate augmented over HR<sub>s</sub> by sympathetic activity. Referring to Fig. 1 of the paper by Robinson et al. (24) it can be seen that HR<sub>s</sub>/HR<sub>s</sub> but not HR<sub>s</sub>/HR was increased during exercise, due to the fact that HR<sub>s</sub> and HR were both increased. HR<sub>s</sub>/HR<sub>s</sub> rather than HR<sub>s</sub>/HR will be the appropriate expression concerning sympathetic activity during exercise. They demonstrated that HR<sub>s</sub> remained essentially constant during exercise and during tilting experiments. Obviously, conclusions concerning the autonomic tone are valid, only if the denominator remains unchanged during the experimental procedures. This condition has been met during
head immersion, since the HR₀ was essentially unchanged (Table 2).

Whether or not baroreceptor activity contributes to the development of diving bradycardia is an unsettled question. Andersen (1) cautioned against the dismissal of baroreceptors as the cause of diving bradycardia, since the baroreceptors contain a rate-sensitive element which responds to rate of pressure change in addition to the level of blood pressure. Our results favor the dismissal of baroreceptors as a contributing factor in diving bradycardia. Bradycardia was evident in the reseretive-treated rats during diving while aortic pressure was actually falling (Fig. 1D and Table 1).

The reduction in cardiac output during diving can be accounted for by the diving bradycardia alone, indicating an unaltered stroke volume. In a recent study by Jones and Holeton (14) in the duck, the stroke volume increased slightly on the average, except in one duck which consistently displayed a reduction in stroke volume during diving. Jones and Holeton reasoned that favorable conditions exist for cardiac pumping during diving since preload (right atrial pressure) is increased, while afterload is unchanged or decreased during diving. But the elevated vagal activity during diving may nullify this favorable condition by suppressing the contractility of the myocardium. It is expected that considerable species variation exists for the stroke output of the ventricles. The present study employing unanesthetized rats indicates a diving response qualitatively and quantitatively similar to that of the diving mammals rather than the nondiving species. It is a potential model for diving studies, as far as the cardiovascular responses are concerned, without the technical difficulties and high expenses often encountered in employing the marine mammals in the laboratory.

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