Cardiorespiratory and Electroencephalographic Responses to Stimulation of the Mesencephalic Tegmentum in Toads, Lizards, and Rats

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Rectangular pulses delivered to the mesencephalic tegmentum were used as central step-like stimuli, and their effects on blood pressure, heart and respiratory rates, and brain waves were tested in toads, lizards, and rats under urethane anesthesia. The delay response was much shorter and the rate of change was faster in rats than in toads or lizards. Response duration was longer in toads and lizards than in rats. Long-lasting error and post-stimulus oscillations were observed in toads and lizards but not in rats. Eighty percent of responses in rats, 53.33% in toads, and only 2.22% in lizards showed adaptation to the stimulus. In toads and lizards the whole variables were systematically affected, whereas in rats 42% of responses were discriminative. Tegmental stimulation induced desynchronization in rats, synchronization in lizards, and had no effect in toads. These results suggest that whereas the cardiovascular control in the rat may act as proportional, derivative, or integrative and in the toad only as proportional, a derivative component becomes apparent in the lizard.

INTRODUCTION

The main source of ambiguity when searching for a clear understanding of the central mechanisms involved in autonomic control in mammals certainly is the extreme complexity of brain circuitry. For this reason, con-

Abbreviations: \( T_{on} \)—time constant "on," \( T_{off} \)—time constant "off," \( t \)—time, \( et \)—transient error, \( rs \)—steady error, \( T_{p} \)—time propagation delay, EEG—electroencephalogram.

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ventional techniques such as electrical stimulation or lesion of nervous centers do not provide the high level of anatomical and functional resolution required.

On the other hand, comparative ideas have proved to be very useful in the formulation of models of neural organization, particularly in fields such as sensory processing (16) and the central control of movement and locomotion (3). The success of this evolutionary approach has stressed the role played by the colonization of new environments in the evolution of central structures controlling somatic functions (9). Because adaptation to new surroundings involves also coping with new requirements for cardiorespiratory adjustments, it is surprising that, except for the paper by Burnstock (2), on the peripheral autonomic system of vertebrates, the comparative scheme has only occasionally been extended to experimental studies on the nervous control of cardiorespiratory functions (4, 5, 11, 12, 14). Obviously, any newly acquired structure or function must have evolved within the framework of preexisting working systems, leading to greater complexity of interconnections both at the level of the central processor and to and from the periphery in successive stages of evolution.

On those grounds we decided to compare aspects of cardiovascular and respiratory control in an amphibian (Bufo arenarum Hensel), a reptile (Tupinambis rufescens), and a mammal (Rattus norvegicus). Of course it is not our intention to claim that all members of a class of vertebrates have the same regulatory mechanisms, or that the examples we chose from each class approximate in any way the common evolutionary link between the classes involved. Inasmuch as vertebrate classes are not arbitrary classification artifacts but groups which share real morphological and functional characteristics, one must expect more intraclass than interclass similarities in the modalities of control, and our chosen species can be taken as representatives of their classes with regard to this particular problem. Comparisons were made after submitting the subjects to a similar central step-like disturbance, e.g., trains of rectangular pulses to the mesencephalic tegmentum and making a transient analysis of both the immediate response to the disturbance and the recovery of initial values after stimulus withdrawal.

MATERIALS AND METHODS

Experiments were carried out at room temperature (25 to 27°C) in Wistar rats (200 to 260 g), tegu lizards (Tupinambis rufescens) (700 to 2100 g), and toads (Bufo arenarum Hensel) (145 to 230 g) anesthetized intraperitoneally with urethane (ethylcarbamate) (1 to 1.5 g/kg). In all cases adult male animals maintained 2 weeks at 25°C were used. The right femoral artery and vein of rats and lizards were cannulated, and the dorsal aorta and the subcutaneous abdominal vein were used in toads.
A large extradural craniectomy was performed to permit electroencephalographic recording and electrode descent for electrical stimulation. The animals were fixed in a stereotaxic apparatus. Arterial pressure measured by a P23 DC Statham transducer, heart rate computed by means of an electronic cardiograph triggered by the R wave, respiratory rate, and the EEG were all recorded on a Grass 7 polygraph. Rectangular pulses to the mesencephalic tegmentum were delivered from a model 161-162 Tektronix unit through concentric electrodes of about 100-μm diameter at the tip. Only one descent was made in each rat according to the following stereotaxic coordinates: lateral, 1.5; anterior, 1.5; and vertical, +2.5, which correspond to the lateral part of the reticular formation (1). Several points of the mesencephalic tegmentum were stimulated in each lizard or toad. The results presented here include only points falling within the shaded area of Fig. 3 as determined a posteriori by histological examination. Trains of rectangular pulses of threshold amplitude (1 to 5 V), 50 Hz, and 1 ms were applied for periods from 10 to 30 s.

Interstimulus interval was never less than 2 min. All brains were perfused with physiologic saline (0.9% in rats and lizards; 0.65% in toads), fixed in 10% formalin, and the paraffin sections were stained with hematoxylin.

Analysis of Results. The step-response technique (8) was systematically applied to pressor, chronotropic, and respiratory responses in the three species (see Table 1). The following criteria and definitions were taken into account: (i) The train of rectangular pulses was considered as a step function. (ii) Delays: latencies were computed as propagation delay, that is, the time interval between the stimulus “on” and the response onset. (iii) Both $T_{on}$ (time constant “on”) and $T_{off}$ (time constant “off”) were measured and compared. $T_{on}$ is the time elapsed until the response reached 63% of its maximum, and $T_{off}$ is the time elapsed from stimulus “off” until the response decreased to 37% of its maximum. (iv) Duration of responses was estimated as the relation $t_1/t_2$, where $t_1$ is the time elapsed from the beginning to the end of the response and $t_2$ is the duration of the stimulus in seconds. (v) Discrimination existed when at least one of the autonomic variables, namely, arterial pressure, heart rate, or respiration, remained unchanged during stimulation. (vi) Ringing involved appearance of damped and irregular fluctuations of the variable, immediately after the stimulus ceased. (vii) Error ($e$) included transient error ($e_t$) when after-stimulus withdrawal lasted four times $T_{off}$ or more before returning to control values; and steady error ($e_s$) when after-stimulus withdrawal lasted longer than 2 min (minimum interstimulus interval) before returning to control values. (viii) Adaptation occurred when the response began to decrease before the stimulus “off.” (ix) Time propagation delay [$T_{PD} = (T_{on} + T_{off})$]
2) was taken as an index of the capability of the system to readjust after disturbances. Statistical differences were analyzed by Student's $t$ test.

**RESULTS**

The results are shown in Tables 1 and 2 and Figs. 1 and 2.

**Rats.** They had four combinations of responses. The most common, 35 of 60 cases (58%), was hypertension with tachycardia and polypnea; 13 of 60 (22%) reactions included blood pressure increase and polypnea, without changes in heart rate. Ten of 60 (17%) showed hypotension plus increase in heart rate; and finally, two of 60 (3%) had isolated hypertension.

**Toads and Lizards.** After tegmental stimulation both species showed hypertension, tachycardia, and polypnea in 100% of cases.

**Delays.** They were significantly shorter in rats than in lizards or toads ($P < 0.001$). No significant differences between toads and lizards were noted.

$T_{on}$. The rate of "on" changes was greater for rats than for the other species ($P < 0.001$). Significant differences were observed also between toads and lizards ($P < 0.001$).

$T_{off}$. The rate of "off" changes in blood pressure was ten times faster in rats than in lizards and about seven times faster than in toads ($P < 0.001$ in both cases). Also, the rates in toads were significantly different from those in lizards ($P < 0.05$).

**Total Duration of Responses and $T_{PD}$.** The lower the value, the better the capacity of the system to adjust to the effects of the disturbance. Both were significantly lower for rats than for the other species ($P < 0.001$).

**Discrimination.** This was observed in 25 of 60 samples (42%) in the rat, whereas in toads and lizards it was never observed.

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**TABLE 1**

Differences in Systolic Blood Pressure and Heart and Respiratory Rate among Rats, Lizards, and Toads

<table>
<thead>
<tr>
<th>Group (and N°)</th>
<th>Systolic blood pressure (mm Hg)</th>
<th>Heart rate (per min)</th>
<th>Respiratory rate (per min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat (10)</td>
<td>106.32 ± 4.86*</td>
<td>376.18 ± 9.73</td>
<td>64.76 ± 2.81</td>
</tr>
<tr>
<td>Tegu lizard (10)</td>
<td>73.35 ± 0.94</td>
<td>41.20 ± 0.85</td>
<td>4.20 ± 0.23</td>
</tr>
<tr>
<td>Toad (10)</td>
<td>37.2 ± 0.43</td>
<td>28.4 ± 0.62</td>
<td>—*c</td>
</tr>
</tbody>
</table>

*a Number of animals.

*b Mean values ± SE.

*c Irregular.
### Table 2

Dynamic Differences in the Cardiorespiratory Responses to Stimulation of the Mesencephalic Tegmentum in Rats, Lizards, and Toads

<table>
<thead>
<tr>
<th>Group</th>
<th>Delay* (s)</th>
<th>T_on* (s)</th>
<th>T_off* (s)</th>
<th>T_PP* (s)</th>
<th>t_e/t_s</th>
<th>Discrimination</th>
<th>Ringingb</th>
<th>Error</th>
<th>Adaptationa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td>0.60 ± 0.04</td>
<td>1.20 ± 0.07</td>
<td>3.76 ± 0.43</td>
<td>2.26 ± 0.12</td>
<td>5.48</td>
<td>7.48</td>
<td>25/60</td>
<td>0/60</td>
<td>16/60</td>
</tr>
<tr>
<td></td>
<td>(57)</td>
<td>(53)</td>
<td>(57)</td>
<td>(58)</td>
<td></td>
<td></td>
<td>(42%)</td>
<td>(0%)</td>
<td>(27%)</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
</tr>
<tr>
<td>Lizards</td>
<td>2.43 ± 0.24</td>
<td>19.30 ± 0.85</td>
<td>28.23</td>
<td>4.05 ± 0.30</td>
<td>28.30</td>
<td>40.5</td>
<td>22/45</td>
<td>5/45</td>
<td>14/45</td>
</tr>
<tr>
<td></td>
<td>(45)</td>
<td>(45)</td>
<td>(44)</td>
<td>(43)</td>
<td></td>
<td></td>
<td>(0%)</td>
<td>(11%)</td>
<td>(11%)</td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.05</td>
<td></td>
<td></td>
<td>(0%)</td>
<td>(0%)</td>
<td>(0%)</td>
</tr>
<tr>
<td>Toads</td>
<td>3.07 ± 0.13</td>
<td>10.36 ± 0.03</td>
<td>29.58 ± 2.68</td>
<td>3.15 ± 0.32</td>
<td>19.97</td>
<td>30.5</td>
<td>21/60</td>
<td>21/60</td>
<td>32/60</td>
</tr>
<tr>
<td></td>
<td>(50)</td>
<td>(60)</td>
<td>(56)</td>
<td>(55)</td>
<td></td>
<td></td>
<td>(35%)</td>
<td>(35%)</td>
<td>(35%)</td>
</tr>
</tbody>
</table>

* These values correspond to the pressor response. See text for further explanation.

b These values correspond to pressor and chronotropic responses.

c Mean values ± SE. Number of samples in parentheses.
Ringing. After tegmental stimulation ringing was observed in toads (21 or 60; 35%) and in lizards (5 of 45; 11%), but never in rats.

Error. The largest percentage of transient error ($e_t$) (22 of 45; 49%) was observed in lizards, an intermediate value was obtained in toads (23 of 60; 38%) and the lowest incidence occurred in rats (16 of 60; 27%). Steady error ($e_s$) was never present in rats, whereas 21 of 60 cases in toads (35%) and 5 of 45 in lizards (11%) showed this accident.

Adaptation. Eighty percent of samples in rats (48 of 60) showed adaptation to the stimulus. Only one of 45 cases (2%) in lizards presented the phenomenon. Toads again gave intermediate values (32 of 60; 53%).

Concerning EEG responses, the tegmental stimulation systematically induced desynchronization in rats, synchronization in lizards, and had no noticeable effect on toads.

DISCUSSION

The technique of step-like central stimulation makes possible both open and closed loop analysis of the cardiorespiratory regulatory system. Transient analysis of the onset of the peripheral responses provides information
on the efferent open loop of autonomic receptors, and transient analysis of the recovery of initial values after stimulus cessation shows the behavior of the system as a whole.

Because one of the main questions we posed was the degree of control of the central processor over the controlled variables, the disturbance we chose was the electrical stimulation of a nervous structure having a crucial role in cardiorespiratory regulation. The main requirement for the validity

![Graphical representation of physiological changes](image)

**Fig. 2.** Comparison of the pressor, chronotropic, respiratory, and EEG changes induced by reticular stimulation in toads, lizards, and rats. In the three cases the duration of the stimulus (black mark between "on" and "off") was 25 s. Note the steady error in blood pressure of toad and lizard, and oscillations in heart rate of lizard, and in blood pressure and heart rate in toads. The close correlation between respiration, hypertension, and heart rate is clear in the toad. Also the differences in the EEG response are noticeable. bp—Arterial blood pressure, hr—heart rate, ecg—electrocardiogram, r—respiration, eeg—electroencephalogram. See text for further explanation.
of our comparison is the homology of the stimulated nervous structure and its involvement in cardiovascular regulations in all the groups under study. The reticular formation of the brain stem satisfactorily meets both requirements as certified by abundant experimental evidence (6, 10, 13-15, 18) and has the additional advantage of being extremely resistant to habituation by electrical stimulation, allowing the comparison of a great number of stimuli without changes in the properties of the system.

The stimulation of the mesencephalic tegmentum elicited peripheral responses four times faster in rats than in lizards and five times faster than in toads (see latencies, Table 2). Assuming a purely efferent mechanism, the difference must be due to nervous conduction velocity, length, and

![Diagram showing the mesencephalic area of the three species where stimulations were made. R—rat, L—lizard, T—toad. GC—Central grey matter, GM—corpus geniculum medialis, CA—cornu Ammonis, FD—fimbria hippocampi, L—nucleus lateralis thalami, RM—formatio reticularis mesencephali, P—pons, T—tectum, A—aqueductus, MPS—mesencephalic periventricular system, nIII—nucleus oculomotoris, FLM—fasciculus longitudinalis medialis, TG—tegmentum mesencephali, NIII—oculomotoris nerve, V—ventriculus mesencephali, TS—torus semicircularis, and NI—nucleus interpeduncularis. The dotted squares indicate the area of electrical stimulation.](image)
number of relays in the pathway and/or vasomotor coupling time. If the experimentally activated neural pathways are the same as those which work under normal conditions, which we believe to be the case, this faster reaction time would give greater ability to the central nervous system of rats to modify the state of cardiorespiratory variables and would enable the animal to "change gears," so to speak, faster than lizards or toads.

It is also worth noting that central stimulation of both lizards and toads affected all the monitored variables simultaneously, whereas responses in rats showed what we define as discrimination, that is, the ability to modify the state of one variable separately. As regards the closed loop analysis, it is known that the circulatory control of mammals can appear to have properties of proportional, proportional plus derivative, or integral control systems (7). The shorter decay time ($T_{off}$) observed in rats suggests the existence of an active braking mechanism in autonomic reactions, whereas the slow decaying of curves in lizards and toads gives support to the idea of passive processes. Also, the lack of steady error or ringing as well as the presence of adaptation of responses indicates the action of a strong negative feedback loop in rats.

In toads the system behaves in a very different way. Variables return slowly to their prestimulus values, and they fluctuate for a time after the stimulus is switched off. We find this suggestive of less effective or even of absent derivative and integral components of the feedback loops compared to the mammalian situation. Lizard responses showed less ringing and steady error than those of toads and more than those of rats, but did not show an equally intermediate performance with respect to recovery times, indicating the possible development of a derivative component in the mechanism involved.

Obviously, the well-defined thermoregulatory differences among rats, toads, and lizards must be considered as the main factors affecting cardiorespiratory reactions to external stimuli. For this reason it was essential to have acclimation as well as a constant temperature during all experiments on the three species. It remains to investigate in what way step-like changes of external as well as of internal temperatures may modify the patterns of cardiorespiratory responses described in this paper.

The EEG synchronization systematically observed in the lizard after tegmental stimulation seems to be in agreement with the results obtained by Tauber et al. (17), who also described synchronization of the EEG during arousal in the lizard Ctenosaura pectinata.

REFERENCES


