BEHAVIORAL AND NEURAL BIOLOGY 41, 77–83 (1984)

BRIEF REPORT

Vasopressin Prolongs Bradycardiac Response during Orientation

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Adult male rats were implanted with transcutaneous ECG electrodes and habituated to a dark chamber with elevated background noise levels. ECG was recorded prior to, immediately after, and 3 min after sudden elimination of background noise. The orienting response to the stimulus offset was accompanied by transient bradycardia. Neither AVP (1 μg/rat) nor oxytocin (1 μg/rat) injected subcutaneously 1 hr prior to testing altered baseline heart rate or the immediate bradycardiac response to stimulus offset. However, AVP, and to a lesser extent oxytocin, prolonged the bradycardia induced by stimulus offset. The results show that neurohypophyseal peptide hormones enhance the cardiovascular component of orienting to stimulus change.

Recent research has shown that neurohypophyseal peptide hormones modify various forms of learned behavior. Post-training vasopressin injections increase active and passive avoidance responding in rats (see van Wimersma Greidanus, Bohus, & de Wied, 1981) and both central and systemic oxytocin injections have been shown to reduce avoidance extinction responding (Bohus, Kovács, & de Wied, 1978; Bohus, Urban, van Wimersma Greidanus, & de Wied, 1978). In addition, vasopressin alters the heart rate changes which accompany changes in behavior. Bradycardia during passive avoidance retention is facilitated by desglycinamide–lysine vasopressin (DG–LVP) (Bohus, 1977), a vasopressin

[1] Basic instrumentation was funded by a grant from the Dr. Saal van Zwanenbergstichting. J. J. Hagan was supported by a fellowship from the Stichting Pharmacologisch Studiefonds and, during preparation of the manuscript, by a grant from the MRC of the United Kingdom to Dr. R. G. M. Morris. J. J. Hagan's present address is: Psychological Laboratory, The University of St. Andrews, South St., St. Andrews, Fife, KY16 9JU, Scotland. The secretarial aid by Mrs. Joke Poelstra-Hiddinga is greatly acknowledged. Send requests for reprints to Dr. Bela Bohus at: Department of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA HAREN, The Netherlands.

0163-1047/84 $3.00

analog which lacks virtually all pressor activity (de Wied, Greven, Lande, & Witter, 1972). The response appears to be vagally rather than sympathetically mediated as both phasic and tonic bradycardia during passive avoidance retention remains intact in rats which have been chemically sympathectomized during infancy (Bohus, de Jong, Provoost, & de Wied, 1970). Orienting to stimulus change or novel stimuli in rats is known also to result in transient bradycardia (Graham & Clifton, 1966) accompanied by general inhibition of motor activity with small postural adjustments required to orient toward the stimulus. Pilot studies showed that in rats habituated to the presence of an auditory tone sudden elimination of the tone caused a transient bradycardia. The bradycardic response to conditioned aversive stimuli is thought to reflect the attentional demands of the situation (Obrist, Webb, Sutterer, & Howard, 1970). If this suggestion is correct then one could expect comparable action of vasopressin in situations involving aversion or orientation. Therefore, we tested vasopressin for its capacity to alter bradycardia induced by orientation to stimulus offset. Oxytocin was also tested to check for the specificity of vasopressin. Doses selected were those shown to be effective in avoidance tests.

The experiments were performed using 24 adult male rats (CPB, TNO, Zeist, The Netherlands) weighing between 160 and 170 g. They were housed four or five to a cage with ad lib food and water in a temperature controlled environment (23 $\pm$ 1°C) on a normal light cycle. Two transcutaneous electrodes were implanted under light ether anesthesia. One was placed between the scapulae and the other in the midback, according to the method previously described (Bohus, 1974). Three days were allowed for recovery before the start of the experiment.

Behavior was studied in a dark chamber (internal dimensions 40 $\times$ 40 $\times$ 40 cm) housed in a sound and light attenuating room. The electrocardiogram (ECG) was monitored using a telemetry system consisting of a miniature FM transmitter (3.5 g; Smith and Nephew Research Ltd., Model SNR102F) attached to a Velcro strap and secured around the rat's thorax. The transmitter was connected to the electrodes using Amphenol plugs. The ECG signal was monitored using a commercially available FM receiver, amplified (Grass Model P5CR) with cut-off frequencies at 7–100 Hz, and recorded using an Analog 7 tape recorder (Philips Instrumental, Ltd.). Data were stored for off-line computer analysis. During recording and analysis the quality of the signal was continuously monitored on an oscilloscope. The transmitter and harness were attached immediately before each session. On Day 1 the rats were confined in the dark compartment for 5 min of adaptation with background noise maintained at a constant level by a mechanical scrambler. ECG was recorded during a 5-min test on the following day. The mechanical scrambler was switched on during the first 2 min but was then switched off, leaving the chamber

in almost total silence for the final 3 min. ECG was recorded for 60 sec on three occasions. The first was *immediately before* the scrambler was switched off (P1), the second was *immediately after* the scrambler was switched off (P2), and finally during the *fifth minute* of confinement, with the scrambler off (P3). Arginine vasopressin (1 μg/rat) or oxytocin (1 μg/rat) were injected subcutaneously 1 hr before the start of testing. Peptides were supplied by Organon, Oss, The Netherlands, and solutions were freshly prepared by adding a single drop of HCL (0.01 *N*) and saline (0.9%) to yield the required dose in a volume of 0.5 ml. Acidified physiological saline was used as a control.

Recorded ECG samples were played through a cardiotachometer which generated a pulse in response to each R wave of the ECG complex. Elapsed time between pulses, the interbeat interval (IBI), was measured by computer (sampling rate = 10 kHz.). IBIs which were less than 100 msec or greater than 219 msec were considered artifactual on physiological grounds and were excluded from further analysis. There were no significant group differences in the number of samples thus excluded. From each sampled period an IBI histogram was computed. Heart rate was expressed as the mean IBI calculated over the entire sampling period. Standard deviations about each sample mean were calculated as an index of the within-sample variablity. Treatment effects on IBI variability were subsequently assessed using this measure (see Table 1). Bradycardia was reflected as an increase in the mean IBI, i.e., increased incidence of

TABLE 1

Variability (expressed as the standard deviation about the mean IBI in each sample period), skewness, and kurtosis in interbeat interval distributions measured prior to (P1), immediately (P2), and 2 min (P3) after a sudden reduction in background noise levels in rats treated with saline, AVP (1 μg/rat sc), or oxytocin (1 μg/rat sc)[a]

Treatment	P1	P2	P3
	Standard deviations		
Saline	10.55 ± 1.06	6.72 ± 1.2	7.57 ± 0.81
AVP	7.96 ± 1.51	5.78 ± 0.72	6.76 ± 1.29
Oxytocin	8.14 ± 0.79	5.43 ± 1.09	9.64 ± 1.93
	Skewness (G1)		
Saline	3.04 ± 0.69	1.53 ± 0.99	5.28 ± 0.45
AVP	4.49 ± 1.05	2.14 ± 1.01	3.85 ± 1.24
Oxytocin	4.27 ± 0.73	2.15 ± 0.97	2.98 ± 0.68
	Kurtosis (log G2 + 3)		
Saline	1.273 ± 0.086	0.817 ± 0.208	1.636 ± 0.062
AVP	1.476 ± 0.179	1.115 ± 0.201	1.295 ± 0.223
Oxytocin	1.559 ± 0.101	1.198 ± 0.154	1.248 ± 0.121

[a] $N = 8$ for all groups. Values (mean ± SEM) were calculated from 60-sec ECG recordings. Peptides were injected 60 min prior to testing.

longer IBIs. To form a more complete statistical description of the IBI histograms and a more exact assessment of the influence of stimulus offset and peptide treatment, indices of skewness (G1) and kurtosis (G2) were also computed. Although more frequently used as descriptive terms the distributions of these indices allows their use in quantitative statistical comparisons. In a symmetrical distribution of IBIs the index of skewness (G1) assumes a value of 0. Positively skewed distributions with a preponderance of long IBIs and relatively fewer short IBIs assume increasingly large positive values of G1. Similarly, increasing peakedness of the distribution about the mean is reflected by increasingly large positive values of G2, whereas flattening of the distribution reduces G2 toward 0. Group histograms were also calculated on the basis of interbeat intervals grouped into bins of 5-msec duration. Frequencies were expressed as a percentage of total intervals in the sample and group means were calculated. Data were analyzed by analysis of variance with Neuman–Keuls test for a posteriori comparisons following a significant F ratio. A probability of $<.05$ was accepted as significant.

Analysis of mean IBIs revealed a significant main effect of observation periods ($F(2, 42) = 16.2$, $p < .0001$) and a significant groups × observation periods interaction ($F(4, 42) = 3.36$, $p = .01$). These data are shown in Fig. 1. In each treatment group the mean IBI significantly increased ($p < .05$) during P2, i.e., the heart rate decreased. There were no significant group differences during P2. In P3 the mean IBI of saline controls was

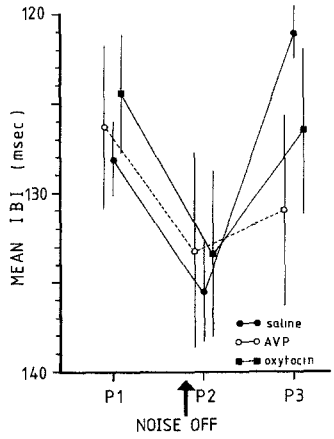

FIG. 1. The effects of pretreatment with AVP (1 μg/rat sc), oxytocin (1 μg/rat sc), or saline on bradycardia induced by a sudden reduction in background noise. Mean (±SEM) interbeat intervals (IBI) were calculated during exposure to elevated background noise (P1) to which the rats had previously been habituated. This was repeated for the first minute following stimulus offset (P2) and again 3 min later (P3). Peptide or saline was injected 1 hr prior to testing. The point of stimulus offset is indicated by the arrow. $N = 8$ for all groups.

significantly lower ($p < .05$) than during P1 or P2. Also during P3 the mean IBI of AVP treated rats was significantly greater than in saline controls ($p < .01$). Oxytocin also tended to increase the mean IBI during P3 although this effect failed to reach significance. Group histograms for P3 data (see Fig. 2) revealed substantial bimodality in interbeat interval distribution after oxytocin, and to a lesser extent, vasopressin. There was no evidence for bimodality within individual records, however, three of the eight oxytocin treated rats had P3 mean IBIs within the range of 140–145 msec whereas the remaining five were in the range of 110–119 msec. Bimodality in the AVP P3 distribution was attributed to one rat with a peak frequency between 165 and 169 msec. IBI variability, expressed as the standard deviation about the mean IBI for each sample period, and the data on skewness and kurtosis within IBI distributions are summarized in Table 1. There were no significant between-group differences in these data although there were clear differences between observation periods in standard deviation ($F(2, 42) = 5.52$, $p = .007$), skewness ($F(2, 42) = 5.52$, $p = .007$), and kurtosis ($F(2, 42) = 6.84$, $p = .002$). Each of these indices was lower during P2 than either during P1 or P3. Thus, there was less within-sample IBI variability and the histograms were relatively flatter, approaching the normal distribution.

A sudden reduction in background noise, in which the rats had been habituated, resulted in a clear bradycardic response. This confirms previous findings (Graham & Clifton, 1966). Reductions in the mean IBI were accompanied by reduced variability in the IBI distributions, which also became less skewed and leptokurtic. These distribution shifts were transient as the increase in mean IBI in saline treated rats was confined to the period immediately after switching off the background noise. Similarly,

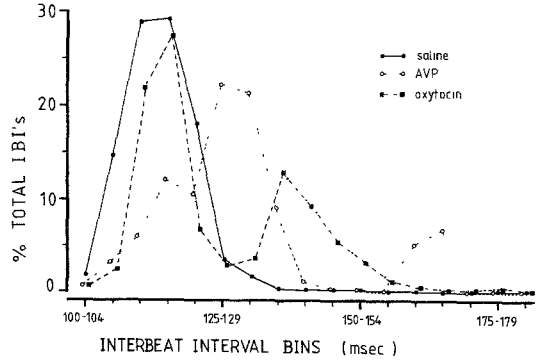

FIG. 2. The effects of saline, AVP (1 μg/rat sc), or oxytocin (1 μg/rat sc) injected 60 min prior to testing, on interbeat intervals (IBI) recorded 3 min after a sudden reduction in background noise (P3). IBIs from each rat were grouped into 5 msec bins then each bin value was calculated as a percentage of the total number of IBIs in the sample period. Group means were calculated for each bin.

the variability, skewness, and kurtosis within the IBI distributions were comparable during P1 and P3. Pretreatment with either AVP or oxytocin had no significant effect on IBI distribution prior to or immediately after the stimulus offset. Although between-subject variability was greater during all observation periods in peptide treated rats (see Fig. 1) the mean IBI was comparable to saline controls during P1 and P2. Therefore neither baseline heart rate nor the animals' responsiveness to stimulus change was altered by the peptides. Assuming close links between cardiac and somatic activity (Obrist et al., 1970) the lack of peptide effects during P1 or P2 argues against any suppressive influence on baseline somatic activity. Our data show that AVP, and to a lesser extent oxytocin, prolonged the bradycardic component of orienting to stimulus offset. Mean IBIs remained elevated 3 min after stimulus offset in both AVP and oxytocin treated rats. Whether this effect is centrally or peripherally mediated is unknown. A central mechanism is suggested by previous experiments which have shown that DG–LVP, a vasopressin analog which lacks virtually all pressor and antidiuretic activity (de Wied et al., 1972) enhances bradycardia during passive avoidance retention (Bohus, 1977). On the other hand we have found a bradycardic influence of centrally injected oxytocin but not AVP during forced exposure to conditioned aversive stimuli (Hagan & Bohus, manuscript submitted for publication). Our unpublished data and those from the present experiment suggest that conditioned aversive stimuli are not a necessary prerequisite for peptide induced facilitation of bradycardia. Bimodality in the oxytocin group data reflects the pronounced bradycardic influence of the peptide in a subset of the test sample. This suggests that the effect of vasopressin cannot be simply generalized for neurohypophyseal hormones. Bimodal effects on avoidance behavior have been reported following peripheral post-training oxytocin injections (Bohus, Urban, et al., 1978). These effects may indicate the existence of two populations which vary either in sensitivity to the peptide or in terms of metabolic degradation following peripheral injections.

Although it seems likely that somatic and cardiovascular activity are inhibited in parallel (Obrist et al., 1970) it remains for further research to establish that this is the case. The data demonstrate that the influence of neurohypophyseal peptides on behavior is not restricted to learning but may be seen in relatively simple situations in which only orientation to stimulus change is required. It remains for future experiments to determine whether or not this represents a physiological action of the peptides and whether facilitation of orientation has any consequences for subsequent cognitive processes.

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