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# Desglycyl-8-Arginine Vasopressin Affects Regional Mouse Brain Cyclic AMP Content

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SCHNEIDER, D. R., B. T. FELT AND H. GOLDMAN. *Desglycyl-8-arginine vasopressin affects regional mouse brain cyclic AMP content.* PHARMAC. BIOCHEM. BEHAV. **16**(1) 139–143, 1982.—The content of cyclic AMP in various regions of the brains of young, adult male Swiss ICR mice, were altered at 60 minutes and 24 hours after a single intraperitoneal administration of desglycyl-8-arginine vasopressin (DGAVP). These time periods are well beyond the reported plasma half-life for this behaviorally active peptide. At 60 minutes, 5 brain regions showed significant elevations. The most notable response was in the septal area, (225%), followed by the thalamus (121%), the olfactory bulb, (144%), and hippocampus, (122%). After 24 hours, cyclic AMP content was significantly elevated in 10 brain regions. Maximal response was found in the hippocampus, (167%), the parietal cortex, (194%), and the occipital cortex, (190%). Other responsive regions included the olfactory bulb, (197%), medulla-pons, (153%), hypothalamus, (152%), septal area, (154%), striatum, (135%), and the frontal cortex, (133%). A substantial response to the injection of a placebo was noted in control animals. In each of the 12 regions examined, the content of cyclic AMP was significantly lower ($p<0.01$ or greater) at 24 hours compared to 1 hour after administration of the placebo. This is consistent with our previous findings of a late effect of the placebo injection. The regional cyclic AMP responses were qualitatively different from those observed after a single injection of ORG 2766, a tri-substituted ACTH (4–9) peptide fragment with different effects on memory. On the basis of the marked increase of cyclic AMP in the septal area, thalamus and olfactory bulb, at 60 minutes, and the prolonged response to DGAVP in multiple brain regions which were found at 24 hours, we conclude that DGAVP triggers serially related biochemical mechanisms which continue to affect the metabolism and function of these areas for long time periods after a single injection.

DGAVP Vasopressin Peptide Mouse Microwave Cyclic AMP Brain regions

THE peptides ACTH/MSH, (adrenocorticotropic hormone/alpha melanocyte stimulating hormone) and vasopressin, together with various synthetic analogs and fragments of such compounds, profoundly influence active and passive avoidance behaviors [8–10, 12], as well as attentional behaviors [2, 4, 23], in rats and in mice [3, 14, 25]. These effects persist for times which are far beyond the detectable plasma half-lives reported for either the ACTH/MSH or vasopressin peptide fragments [34]. Whether these substances exert biological and behavioral actions by means of direct binding onto cellular receptors, or by the modulation of cellular biochemical events is unknown.

Specific effects of ACTH/MSH and vasopressin fragments in selected brain regions and microregions have been reported from several laboratories [9, 11, 16, 26, 27, 31]. For example, the tri-substituted ACTH (4–9) derivative, ORG 2766, has been localized to specific septal and thalamic nuclei by Verhoef and collegues [35]. Vasopressin, (AVP), and its desglycyl-8-arginine derivative, (DGAVP), have been found to alter the turnover of norepinephrine in the parafascicular nucleus of the thalamus and to have action in the hippocampus [30]. These findings suggest: (1) that the pituitary peptides or their fragments may be normally involved in CNS functions related to memory; (2) that the sites of action through which these compounds mediate behavioral responses is probably contained in distinct brain microregions; and, (3) that the actions of these peptides probably includes the modification of numerous intracellular biochemical mechanisms.

Biochemically, one effect of several of the behaviorally active peptides, including ORG 2766, alpha-MSH, and MIF-1, involves adenylate cyclase systems. This action has been shown both *in vitro* and *in vivo* [5, 19, 36]. In prior studies by this laboratory, a single IP injection of the potent analog, ORG 2766, was found to alter the cyclic AMP content *in vivo*, in 9 of 12 brain regions of the male mouse during the first 60 minutes after injection [26]. Activation of the tissue adenylate cyclase systems was maximal in frontal and parietal cortices, the midbrain, septal area, and the thalamus. Moreover, changes in cyclic AMP content in the midbrain, the hippocampus and the parietal cortex were still detectable as late as 24 hours following the peptide injection. In contrast to the male, content of cyclic AMP in brain regions of the female mouse were unchanged from controls 30 minutes following peptide injection, but were changed in 9 of 12 regions after 24 hours [27].

In the present study, we report on the early and long term changes of regional brain content of cyclic AMP in the young adult male mouse following a single IP injection of desglycyl-8-arginine vasopressin, (DGAVP). This analog re-

tains all of the behavioral effects but little, if any, of the pituitary, cardiovascular or renal actions of vasopressin. Our findings suggest that the pattern of regional cerebral changes in cyclic AMP elicited by ORG 2766 and DGAVP are qualitatively different, in terms of: (1) the regions which respond to the peptide, (2) the direction of the tissue cyclic AMP content changes which occur, and, (3) the time course of the changes in the respective tissues which are affected.

## METHOD

### *Animal Handling and Drug Injections*

Group-housed adult male, Swiss ICR mice, weighing 25–30 g, were maintained on a 12 hour light-dark schedule. Animals were randomized and brought to the laboratory 24 hours before each experiment. For each experimental protocol, animals received, IP, a 0.1 ml volume containing 10 μg/ml (40 μg/kg) of DGAVP, in a 0.01 N acetic acid-isotonic saline vehicle, pH 3.2. Control animals received only vehicle injections. After the handling and injection procedure, animals were returned to their original group cages. All animals were injected at 9:00 a.m. Animals which were examined at 60 minutes were sacrificed at 10:00 a.m.; animals examined at 24 hours were sacrificed at 9:00 a.m. the following day.

### *Animal Sacrifice and Tissue Handling*

Individual animals were removed from the group cage and placed on a platform adjacent to a WR-430 microwave waveguide-chamber. Animal sacrifice was performed as previously described, using a focussed 3.5 kW, 2450 MHz microwave energy radiation (MWR) for 350 msec, delivered to the head of the conscious, unrestrained and, presumably, unstressed animal [27]. Brain temperatures were sampled using a needle thermocouple probe placed into the core of the brain at about the location of the hypothalamus. Data from animals were accepted for these experiments if brain core temperatures were between 83° and 95°C within 5–8 seconds following MWR. When cooled to room temperature, each brain was removed and dissected into 12 regions according to the guidelines of Glowinksi and Iversen [15]. Dissected tissues were placed into freshly prepared 7% trichloroacetic acid, and were processed for measurements of cyclic AMP. Protein assay for all brain tissues was performed using the method of Lowry [21].

### *Cyclic Nucleotide Analysis and Data Reduction*

Assay of cyclic AMP was performed using the double antibody radioimmunoassay system described by Steiner *et al.* [28]. Results of the assays were computed by a packaged RIANAL program available at the Wayne State University Computer Services Center [13]. Data were further evaluated by one-way and multiple analysis of variance tests, and the Neuman-Keuls test using available packaged computer programs [1,37].

## RESULTS

### *Cyclic AMP and Desglycyl-8-Arginine Vasopressin*

The early (60 min) effects of DGAVP on regional cyclic AMP content in the young male mouse are summarized in Table 1. Of the twelve brain regions examined, the content of cyclic AMP was significantly changed in five areas, including highly significant increases in the septal area, (225%), olfactory bulb, (145%), hypothalamus, (128%), hippocampus, (122%), and thalamus (121%).

By twenty-four hours, when compared to placebo injected animals, cyclic AMP content was significantly increased in 10 of the 12 brain regions examined, Table 2. Responding tissues include the olfactory bulb (197%), parietal cortex, (194%), occipital cortex, (190%), hippocampus, (168%), septal area (153%), medulla-pons (153%), hypothalamus, (152%), thalamus, (135%), striatum, (135%), and the frontal cortex, (133%).

### *Placebo Findings*

At the 60 minute time interval, the *in vivo* content of cyclic AMP in each of the 12 brain regions examined was determined to be within limits previously reported by others [17]. For identically injected animals examined 24 hours after the placebo injection however, the content of cyclic AMP was found to be significantly decreased from the 60 minutes placebo treatment group ($p<0.01$ or greater) for each brain region, Table 2.

## DISCUSSION

In conscious unrestrained male mice the regions of the brain in which the content of cyclic AMP is affected by the administration of the DGAVP analog of vasopressin are different from those affected by the N-terminal tri-substituted analog of ACTH (4–9), ORG 2766 [26]. Furthermore, the time course of changes in regional content of brain cyclic AMP is different. Because ORG 2766 affects short term memory and attentional processes, it was expected that this compound would probably be characterized by direct binding, or by changes in rapidly modulated cellular systems. On the other hand, a protracted behavioral action of DGAVP is suggested by the extended neurochemical responses in the hippocampus and thalamus over a period of 24 hr (Table 2).

The triggering by DGAVP of mechanisms which affect adenylate cyclase systems in several regions of the brain resembles the functional action of other hormones and neurotransmitters. Such changes in levels of intracellular cyclic AMP have been noted to mediate neuronal responsiveness, changes in the rates of ion flux, alterations in neuronal membrane permeability, and changes in the turnover of specific transmitter substances [7, 22, 24].

As shown in Table 1, the neurochemical actions of DGAVP after 60 minutes are most intense in the septal area, followed by the olfactory bulb, the hypothalamus, hippocampus and the thalamus. These early actions of are presumed to be related to peptide interactions on postsynaptic neuronal membrane receptors. It is unknown whether these effects are the result of direct or indirect stimulation of specific receptor sites. More than a doubling of the cyclic AMP content in the septal area strongly suggests that DGAVP, like ORG 2766 [35], could have direct actions in selected nuclei of this region.

This study also indicates that response to a single injection of the DGAVP fragment continues for at least 24 hours in 10 of the 12 brain regions examined. This finding is of importance due to the limited (4–5 minute) half-life reported for this peptide [33]. Extended actions of the peptide on adenylate cyclase systems throughout the brain reduce the likelihood of mech-

TABLE 1

CYCLIC AMP CONTENT OF MOUSE BRAIN REGIONS FOLLOWING DGAVP ADMINISTRATION: MALE MICE, 60 MINUTES

| Brain Region | Placebo | DGAVP |
|---|---|---|
| Olfactory Bulb | 9.92 ± 0.8 | 14.33 ± 1.6† |
| Cerebellum | 9.21 ± 0.5 | 8.87 ± 0.8 |
| Medulla-Pons | 12.22 ± 0.8 | 15.06 ± 1.9 |
| Midbrain | 10.98 ± 0.4 | 11.05 ± 0.6 |
| Hypothalamus | 23.41 ± 1.3 | 30.06 ± 3.3* |
| Septal Area | 27.81 ± 2.2 | 63.95 ± 4.4‡ |
| Thalamus | 14.11 ± 0.6 | 17.13 ± 0.8† |
| Hippocampus | 12.17 ± 0.7 | 14.85 ± 0.9* |
| Striatum | 15.36 ± 0.9 | 14.71 ± 0.9 |
| Frontal Cortex | 16.69 ± 1.1 | 20.45 ± 2.7 |
| Parietal Cortex | 17.21 ± 1.0 | 20.21 ± 1.2 |
| Occipital Cortex | 13.21 ± 0.9 | 15.56 ± 1.3 |
| Number/Group | 19 | 9 |

All data expressed as picomoles cyclic AMP/mg protein; Mean ± S.E.M.

*$p<0.03$; †$p<0.01$; ‡$p<0.0001$.

TABLE 2

CYCLIC AMP CONTENT OF MOUSE BRAIN REGIONS FOLLOWING DGAVP ADMINISTRATION: MALE MICE, 24 HOURS

| Brain Region | Placebo | DGAVP |
|---|---|---|
| Olfactory Bulb | 6.65 ± 0.7† | 13.10 ± 1.9‡ |
| Cerebellum | 6.21 ± 0.6§ | 8.03 ± 0.8 |
| Medulla-Pons | 7.48 ± 0.9§ | 11.43 ± 0.7‡ |
| Midbrain | 8.48 ± 0.4§ | 8.51 ± 0.4 |
| Hypothalamus | 13.72 ± 1.4¶ | 20.90 ± 0.9‡ |
| Septal Area | 17.69 ± 1.8† | 27.20 ± 2.4‡ |
| Thalamus | 8.13 ± 0.4¶ | 10.99 ± 0.8‡ |
| Hippocampus | 6.46 ± 0.6¶ | 10.84 ± 0.5¶ |
| Striatum | 9.67 ± 0.8¶ | 13.02 ± 0.7‡ |
| Frontal Cortex | 12.18 ± 1.0† | 16.17 ± 1.6* |
| Parietal Cortex | 8.88 ± 1.1¶ | 17.23 ± 1.1¶ |
| Occipital Cortex | 8.25 ± 1.0§ | 15.67 ± 1.6§ |
| Number/Group | 11 | 10 |

All data expressed as picomoles cyclic AMP/mg protein; Mean ± S.E.M.

*=$p<0.03$; †=$p<0.01$; ‡=$p<0.005$; §=$p<0.001$; ¶=$p<0.0001$.

Significance following DGAVP treated animal data relates differences between the placebo and treatment at 24 hours. Significance following placebo treatment relates differences between placebo treatment at 60 minutes and 24 hours after injection.

anisms requiring direct involvement of cellular receptors. Such effects may be the result of biochemical events triggered by DGAVP following receptor actions or of the uptake of peptide in selected microregions of the brain [4,30].

*Placebo Effects*

A consistent finding of this study shows that tissue cyclic AMP content is significantly decreased ($p<0.01$ or greater), in each of the brain regions examined 24 hours following an i.p. injection compared to placebo animals analyzed after 60 minutes. This observation replicates findings previously reported by us in male and female mice [26,27]. In the present study, regional cyclic AMP concentrations at 24 hours were 52–77% of those seen at 60 minutes.

These observations have implications for a variety of biochemical, pharmacological and behavioral studies involving animal handing and/or injection protocols. To our knowledge, *in vivo* cyclic AMP changes have only been described in diurnal rhythm urinary excretions, and consist of relatively small changes throughout any 24 hour period [5]. The biochemical effects of DGAVP suggest that this substance may normalize selected biochemical responses (such as the level of cyclic AMP in brain regions), triggered by injection or handling. In another comparison, if 60 minute placebo animals are compared to the 24 hour peptide treated animals, only 4 regions are peptide responsive, including the olfactory bulb, midbrain, thalamus and striatum. We interpret this data to suggest that DGAVP may block extended stress-induced actions within the brain, which may result from prolonged decreases in tissue cyclic AMP.

The long-term consequences of experimental stress as well as the mode and speed of killing have not been well controlled in most reports concerned with either the behavioral or biochemical effects of pituitary hormones and their fragments. We believe that our reproducible observations of effects of placebo injections, as well as time-dependent regional changes in brain cyclic AMP were made possible by the use of high-power, focussed microwave energy which was rapid in onset, and by relatively stress-free procedures to position each animal within the microwave waveguide. At the present time, the duration of the placebo effect is unknown. Also unknown is whether this effect may be duplicated by other routes of administration.

*Commentary*

The influence of DGAVP on regional brain adenylate cyclase systems at early times following an IP injection, and to normalize these same systems at later times has not been previously reported. Others have observed that the synthesis and metabolism of norepinephrine is increased following the administration of DGAVP [29]. The stimulus for such neurochemical changes could be linked to intermediate compounds activated or stimulated by DGAVP at a remote site of binding. At this time, there have been no reports of the availability of a radiolabeled or fluorescent probe with which to determine either the localization or the fate of DGAVP or any possible DGAVP metabolites. Careful determinations of the localization of DGAVP binding in responsive brain regions should reveal additional important and pertinent information regarding the locations and some functional mechanisms of the central actions of this peptide.

In prior behavioral studies, the microinjection of various behaviorally active peptides or peptide fragments, such as ACTH, AVP and oxytocin into local brain areas, has been

shown to affect memory and behavioral extinction differently [20]. The results of our *in vivo* studies with mice now indicate tissue region and microregions of the brain which are neurochemically responsive to the of actions of such compounds. Such findings suggest a possible neurochemical basis for investigating the time-dependent effects of peptides on behavior.

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