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Memory deficit in rats with hereditary diabetes insipidus

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Rats in which the posterior lobe of the pituitary, the storage organ for vasopressin and oxytocin, is removed are markedly less resistant to extinction of a shuttle box avoidance response than sham-operated control animals. Treatment with Pitressin or lysine-8-vasopressin (LVP) restores resistance to extinction in posterior lobectomized rats[9]. In addition, LVP ameliorates impaired avoidance learning of a shuttle box avoidance response in hypophysectomized rats[3], facilitates passive avoidance learning[1], increases resistance to extinction of a shuttle box avoidance[12] or a pole jumping avoidance response[10,13] in intact rats. Similar effects were found with desglycinamide-8-lysine vasopressin (DG-LVP), a peptide isolated from hog pituitary material[6]. This peptide lacks almost all classical endocrine activities due to the absence of the C-terminal glycinamide[14]. The influence of vasopressin and analogues is of a long term nature and lasts much longer; this can be accounted for by the presence of the peptide in the organism[3,10]. Central application of LVP in amounts which are ineffective when given systemically, in particular in the posterior thalamic area and in the cerebrospinal fluid, effectively increases resistance to extinction of a pole jumping avoidance response[13], indicating that the effect of vasopressin is of central origin, and not mediated by peripheral hormonal influences. Physiological evidence for a role of vasopressin in memory processes, however, obtained from subjects without endogenous vasopressin synthesis, has never been provided.

Some years ago, a strain of rats was developed with hereditary hypothalamic diabetes insipidus (Brattleboro strain)[7,8]. Derived from a Long Evans strain (Rockland colony), these animals lack the ability to synthesize vasopressin[7,8]. This disease appears to be due to autosomal recessive genes at a single pair of loci[8]. The availability of these rats and their heterozygous litter mates, which have a relatively normal water metabolism, made it possible to study the memory function of rats in the absence of vasopressin.

Male Brattleboro SPF rats, weighing between 150 and 170 g, obtained from Central Breeding Laboratory TNO, Zeist, Holland, were used. They were differentiated for homozygous and heterozygous diabetes insipidus by measuring 24 h water intake and vasopressin content of posterior pituitaries. Homozygous rats drank 71 ± 3.3 ml/24 h/100 g (n = 10), while heterozygous animals drank 17.0 ± 0.6 ml/24

TABLE I

PASSIVE AVOIDANCE BEHAVIOR IN RATS WITH HEREDITARY DIABETES INSIPIDUS; COMPARISON BETWEEN HOMOZYGOUS AND HETEROZYGOUS RATS. EFFECT OF ARGININE-8-VASOPRESSIN (AVP) AND DESGLYCINAMIDE LYSINE-8-VASOPRESSIN (DG-LVP)

Group	*n*	*Shock*		*Median avoidance latency in sec*					
		mA	*sec*	*Before shock*	*After shock*				
					0 h	*3 h*	*24 h*	*48 h*	*120 h*
Heterozygous	6	0.00	—	6.0	—	—	8.5	6.0	8.5
	8	0.25	3	7.5	—	—	8.5	21.0	12.0
	8	0.50	3	9.0	—	—	30.5	143.0*	101.5*
	8	1.00	3	8.0	—	—	300.0**	300.0**	300.0**
	6	1.00	10	5.0	—	—	300.0**	300.0**	300.0**
	8	0.00	—	9.0	9.5	—	—	—	—
	8	1.00	3	8.5	300.0**	—	—	—	—
	8	0.00	—	9.0	—	10.5	—	—	—
	8	1.00	3	7.5	—	300.0**	—	—	—
Homozygous	6	0.00	—	4.5	—	—	8.0	8.5	8.0
	6	0.25	3	9.0	—	—	11.5	13.0	20.0
	7	0.50	3	9.0	—	—	34.0	37.5	33.0
	7	1.00	3	10.0	—	—	34.0	42.0	40.0
	6	1.00	10	6.0	—	—	28.5	12.5	9.0
	8	0.00	—	7.5	9.5	—	—	—	—
	6	1.00	3	7.0	287.5**	—	—	—	—
	8	0.00	—	8.0	—	10.0	—	—	—
	6	1.00	3	7.5	—	25.5**	—	—	—
DG-LVP 1 μg§	6	0.00	—	8.0	—	—	11.0	11.0	10.0
DG-LVP 1 μg§	8	1.00	3	7.5	—	—	253.5**	300.0**	300.0**
AVP 1 μg§	7	1.00	3	6.0	—	—	300.0**	300.0**	260.0**

* $P < 0.05$.
** $P < 0.01$.
§ dose per rat.

h/100 g (n = 10). The latter animals did not differ in water intake from our Wistar strain (17.6 $\pm$ 3.0 ml/24 h/100 g (n = 6)). Vasopressin content of posterior pituitary lobes was determined in the urethanized Dibenzyline treated rat[4]. No detectable levels of vasopressin were found in the posterior lobe of homozygous rats. Vasopressin content of heterozygous animals amounted to 55.7 $\pm$ 6.3 mU/posterior lobe (n = 8), which is significantly lower than that of our Wistar strain (106.0 $\pm$ 19.5) (n = 8).

Memory was studied in a one-trial step-through passive avoidance test[2]. The apparatus consisted of a wire mesh illuminated elevated runway connected with a dark box equipped with a grid floor. When placed on the runway, animals enter the dark box within a few seconds. Rats are pretrained to enter by giving one trial on day I and 3 trials on day II. Immediately after entering the dark box during the third trial on day II, animals received a 3-sec unavoidable scrambled footshock of various intensities through the grid floor. Latency to enter the dark box (to a maximum of 5 min) was measured 24, 48 and 120 h later.

Retention latency increased with the intensity of the shock in heterozygous rats.

Animals exposed for 3 sec to 1 mA shock failed to re-enter the shock compartment during the retention trials (Table I). The response latencies of these rats after shock were also significantly longer than the preshock latencies (Wilcoxon test) at a shock intensity of 0.50 mA. On the other hand, none of the homozygous rats exhibited passive avoidance behavior. The highest shock intensities increased avoidance latencies slightly but these were not significantly different from preshock scores. It is possible that animals would show memory if shock levels or shock duration would be increased. However, exposure to 1 mA for 10 sec also did not significantly increase avoidance latency in homozygous animals (Table I). A single subcutaneous injection of 1 μg of arginine vasopressin (AVP) or 1 μg of DG-LVP per rat, immediately following the learning trial of homozygous rats exposed to 1.0 mA of shock, restored avoidance latency towards values indistinguishable from those of heterozygous rats exposed to a similar shock intensity. The difference between treated and control animals (Mann–Whitney's U-test) was significant for both AVP ($P < 0.01$) and DG-LVP ($P < 0.05$). No effect of DG-LVP in non-shocked rats was found (Table I).

These results show that rats with an inborn error in the synthesis of vasopressin have a serious deficit in memory processes relative to heterozygous rats which do exhibit avoidance learning. Repeated retention tests did not result in passive avoidance behavior (Table I) and even exposure to shock of much longer duration, which very effectively increased avoidance latency[2], did not elicit memory expression in homozygous rats. Thus, in a one trial learning situation, in the absence of vasopressin, a complete impairment of memory is found 24 h after aversive stimulation. To investigate whether learning or memory processes are involved, groups of heterozygous and homozygous rats exposed to a shock intensity of 1.0 mA were tested for retention either immediately or 3 h after the learning trial. Heterozygous animals at both intervals showed a maximum avoidance latency of 300 sec. Avoidance latency in homozygous rats was near maximal if tested immediately after the learning trial. However, 3 h after exposure to shock, the latency was markedly less, although still significantly different from preshock values (Table I). Since a complete impairment of avoidance behavior was found 24 h after the learning trial, it follows that memory rather than learning processes are disrupted in homozygous rats. It might, however, be possible that multiple learning trials could have elicited memory formation. This could be revealed by the use of multi-trial learning procedures which are at present under investigation.

Since the difference in avoidance behavior might have been due to a difference in shock sensitivity between homozygous and heterozygous animals, response behavior of the two groups of rats to unescapable electric footshock (EFS) was determined[5]. Two sets of 11 shock intensities were used. Each shock was presented for 1 sec and the interval between shocks was 20 sec. During each presentation of shock the following parameters were recorded: no response, flinch, and jerk. Values of shock threshold for a response were calculated as the mean of the lowest intensities to which the rats made a response. No differences in responsiveness were found between groups, nor was a single injection of 1 μg DG-LVP per rat capable of affecting the threshold for flinch or jerk in homozygous or heterozygous rats. Studies in an open

field also failed to indicate a difference between heterozygous and homozygous rats in ambulation, grooming, rearing and defecation during a 3 min exposure.

The difference between homozygous rats and their heterozygous litter mates is the absence of vasopressin. Thus, for the first time, the physiological significance of vasopressin in memory processes is demonstrated. The result of the deficit of homozygous rats could be a change in arousal level, in attention, in retrieval processes, etc. Information on these points is not available at present. Observations in Wistar strain rats[10,13] show that a single injection of vasopressin given within 1 h either before or after an acquisition or extinction session results in a long term preservation of the avoidance response. This suggests that memory processes are involved. In fact, a single injection of the rat's vasopressin, AVP or of the vasopressin analogue DG-LVP normalized avoidance learning of homozygous diabetes insipidus rats. The latter peptide hardly affects water diuresis[14]. Thus, the ameliorating effect cannot be due to the restoration of any disturbance in water metabolism of diabetes insipidus animals. Nor can these effects be mediated by other hormonal effects of vasopressin i.c. blood pressure, oxytocic, corticotrophin releasing activity, because these also are virtually absent in DG-LVP[14]. Since pituitary–adrenal system hormones have a 'short term' effect on active and passive avoidance behavior[11], the results also can not be explained on the basis of a reduced pituitary–adrenal response, which has been reported to occur in homozygous rats[15].

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