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Evidence for Antipyretic Vasopressinergic Pathways and Their Modulation by Noradrenergic Afferents

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Summary

The experimental evidence for the antipyretic action of arginine vasopressin (AVP) in guinea-pigs can be summarized as follows: The febrile response to a bacterial pyrogen can be reduced by a microinfusions of exogenous AVP into the ventral septal area of the limbic system. Immunohistochemical studies indicate increased activity of AVP terminals in the ventral septal area (VSA) and in parvocellular AVP neurones of the hypothalamic paraventricular nucleus (PVN) in several stressful situations accompanied by reduced febrile responses (late stage of pregnancy, immobilization, cold adaptation, osmotic stimulation). Also the peripheral and/or central release of AVP measured in some of these situations is increased. Electrical stimulation of the PVN suppresses fever; this suppression can, at least partly, be cancelled by simultaneous intraseptal application of the vasopressinergic V1 receptor antagonist. The documented AVP pathways from the PVN to the septum receive noradrenergic afferents from the lower brainstem. Chronic destruction of these afferents by microinjections of 6-hydroxydopamine (6-OHDA) significantly reduced the fever responses to pyrogen application, while microinfusion of noradrenaline (NA) enhances the fever reaction.

Key words

Arginine – Vasopressin – Antipyresis – Stress – 6-hydroxydopamine – Paraventricular nucleus

Introduction

The febrile increase of body temperature can be regarded as part of a complex host response to infection accompanying the activation of the immune system (Kluger, 1991). Since the body temperature rarely exceeds 41 °C during fever, it has been proposed that the elevation in body temperature is limited and sometimes even prevented by the action of endogenous antipyretic substances liberated within the brain during fever. Such an antipyretic function has been ascribed to AVP (Cooper *et al.* 1979), the adrenocorticotropic hormone (Lipton and Glyn 1980), or its fragments the melanocyte stimulating hormones, the α-MSH (Samson *et al.* 1981) and the γ-MSH (Bock *et al.* 1991). The antipyretically active site has been identified in the VSA (for recent reviews cf. Kasting 1989, Zeisberger 1990). The AVP afferents to the VSA originate from different hypothalamic nuclei in guinea-pigs to a large extent from parvocellular neurones in the PVN; (for experimental evidence see Zeisberger 1991). In the present paper, we try to summarize the evidence for the antipyretic action of AVP from foregoing experiments in guinea-pigs in which we investigated the febrile responses to pyrogen application under the influence of different physiological, physical or neurochemical stimuli able to activate vasopressinergic pathways within the central nervous system. The PVN and other hypothalamic structures participating in endogenous antipyresis and further important vegetative functions are influenced by aminergic afferents from the lower brain stem. Especially the thermoregulatory thresholds, the mean body temperatures, at which thermogenetic heat production and mechanisms of heat dissipation are activated,

are significantly modulated by aminergic afferents to thermointegrative structures in the anterior hypothalamus (Brück and Zeisberger 1990). Fever is regarded not as a failure of the thermoregulatory system, but rather as a regulated shift of body temperature to a higher level. Antipyretic mechanisms seem to reset the febrile shift of the thermoregulatory thresholds. We therefore tried to elucidate the noradrenergic input into the PVN as a factor in the control of the activity of antipyretic pathways and investigated whether the febrile response to bacterial endotoxin could be altered by manipulation of the noradrenergic input into the PVN.

Methods

The febrile response to intramuscular injections of bacterial lipopolysaccharide (E. coli, 20 μg/kg) was tested in different groups of guinea pigs either as controls or under the influence of several stimuli (see below). The fever responses were evaluated as colonic temperature/time curves for 6 h after the pyrogen injection. The integrated area between the temperature/time curves of febrile and afebrile animals, the fever index, was expressed in °C/h (for 6 h). In order to compare the results of different experimental series, the control fever index was set as 100 %, and the fever indices under the influence of different stimuli were expressed in % of the control value. The colonic temperature was measured every 30 min by inserting a thin plastic coated thermocouple 6 cm beyond the anus. This procedure did not cause excitement or any apparent alteration in body temperature of the animals. Cold adaptation was performed for three weeks in a climatic chamber at an ambient temperature of 5 °C. Osmotic stimulation was achieved by 24 h water deprivation. The animals were immobilized in a sitting position by binding their extremities to a plexiglass plate. In two experimental series, the fever response was tested in the last stage of pregnancy (1 day prepartum). Blood plasma samples were collected *via* catheters chronically implanted into the left carotid artery one week before the start of the experiments. Microinfusions into different central nervous structures or pushpull perfusions of the ventral septal area in the limbic system were performed by use of stereotaxically implanted cannulae. PVN neurones were electrically stimulated by implanted electrodes. Destruction of noradrenergic afferents to the PVN was achieved by microinjections of 6-OHDA into the hypothalamic area within the PVN. Vasopressinergic neurones, fibres and nerve terminals were visualized using immunohistochemical methods. The amounts of AVP were measured in blood plasma samples or in pushpull perfusates of the limbic septal area by use of radioimmunoassay (courtesy of Prof. Dr. E. Simon, MPI für Physiologische und Klinische Forschung, Bad Nauheim, F.R.G.). Fever indices as well as the amounts of AVP in blood plasma or pushpull perfusates were statistically compared by Student's t-tests. For further details on the methods see the following papers: Fever induction and measurements (Zeisberger *et al.* 1981, Cooper *et al.* 1988) stereotaxic technique, microinjections and microinfusions (Zeisberger 1989, Unger *et al.* 1991), cold adaptation, osmotic stimulation and blood sampling (Zeisberger *et al.* 1988, Zeisberger and Roth 1989), immunohistochemistry (Merker *et al.* 1980, 1989), AVP-RIA (Gray and Simon 1983), electrical stimulation and chemical manipulation of the PVN (Unger *et al.* 1991).

Results

Fig. 1 summarizes the results of several experimental series, in which the febrile response to pyrogen application was studied under the influence of different stimuli and compared to unstimulated control animals. It is obvious that an intraseptal microinfusion of AVP (9.6 g AVP dissolved in 36 μl saline and infused during 6 h) reduces the febrile response to 22 % of the control value ($p<0.01$).

Several physiological situations, such as the last day of pregnancy ($p<0.001$), dehydration ($p<0.01$), cold adaptation ($p<0.05$) or immobilization ($p<0.001$) are also accompanied by a significant reduction of the fever reaction after pyrogen application. In immobilized guinea-pigs, the fever response is not only reduced, but the animals become hypothermic

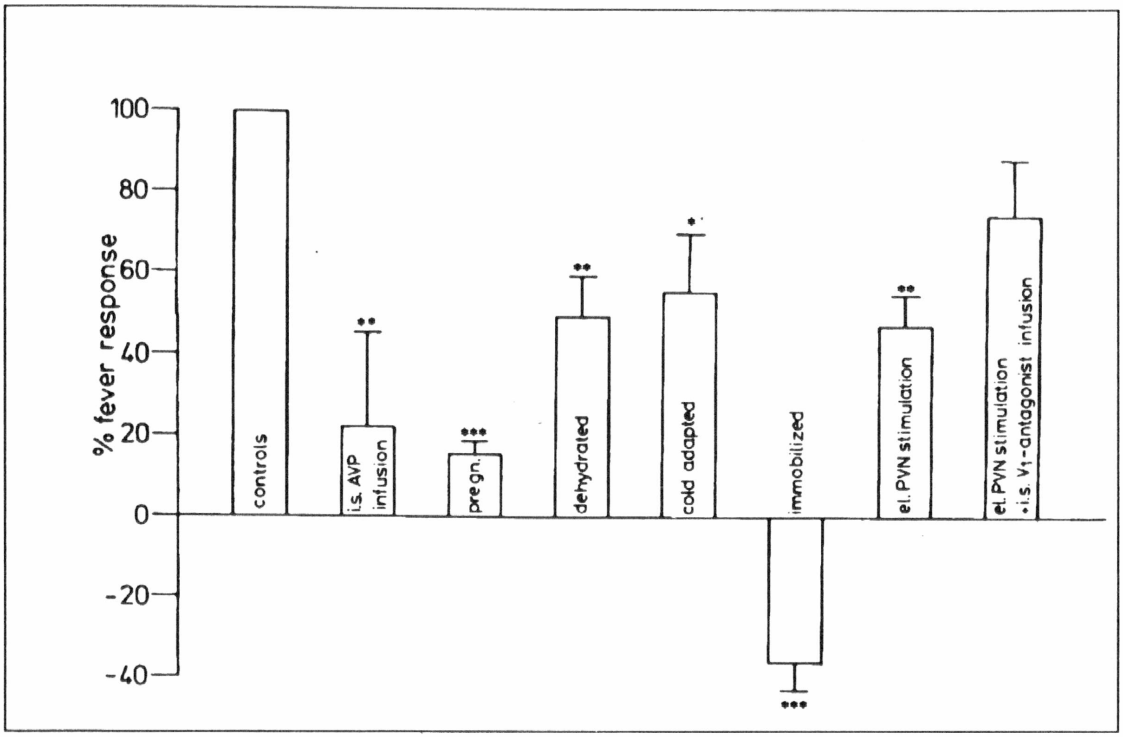

Fig. 1

Comparison of the mean fever indices from different groups of guinea-pigs (n = 6-20) as controls or under the influence of different stimuli. To compare data of several experimental series, the control fever index was always set as 100 % fever response (i.s. = intraseptal; pregn. = pregnant; el. = electrical; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

(cf. column 6 in Fig. 1) so that the fever index calculated for 6 h attains a negative value. In these 4 physiological situations, we examined vasopressinergic brain structures immunohistochemically and detected changes, compared to the controls, indicating increased activity of AVP terminals in the ventral part of the limbic septal area as well as of the parvocellular pool of vasopressinergic neurones in the PVN (not shown; cf. Merker *et al.* 1989). The results indicate that not only exogenous AVP acts as an antipyretic, but that antipyretic reactions can also be caused by activation of endogenous vasopressinergic pathways from the hypothalamic PVN to the limbic septal area. This assumption is also confirmed by the results presented in columns 7 and 8 in Fig. 1. Electrical stimulation of AVP neurones within the PVN significantly reduces the fever reaction ($p<0.01$). The fever reduction by electrical PVN stimulation can, however, be cancelled to a great extent by simultaneous intraseptal application of a vasopressinergic V1-receptor antagonist.

The immunohistochemical changes indicating increased activity of AVP pathways in the brain are found in situations characterized by reduced febrile responses to pyrogens. In some of these situations we also measured central and/or peripheral release of AVP and compared it to control values. The results of these measurements are summarized in Fig. 2. As is obvious from the upper part of Fig 2, the release of AVP into the circulation is significantly increased in dehydrated ($p<0.001$), cold adapted ($p<0.001$) and immobilized ($p<0.05$) guinea-pigs when compared to control animals. The levels in pushpull perfusates of the limbic septal area are also significantly higher in dehydrated ($p<0.01$) and immobilized ($p<0.05$) than in control animals. Pushpull samples of cold adapted guinea-pigs have not yet been collected and analyzed. It seems that physiological situations characterized by reduced febrile responses are accompanied by increased peripheral and central release of AVP. The highest observed levels of AVP are,

however, not correlated to the lowest fever reactions. This becomes obvious by comparison of dehydrated and immobilized animals.

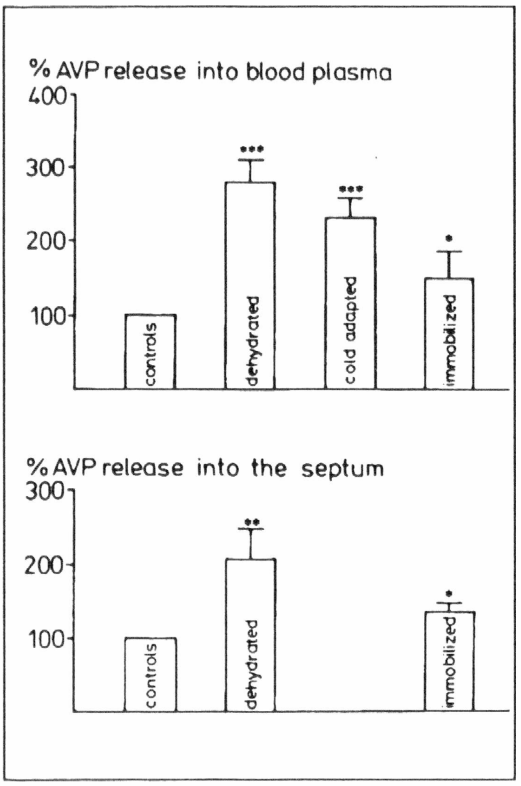

Fig. 2
Comparison of the release of AVP into the circulation (upper part) or into the limbic septal area (lower part) collected from different groups of guinea-pigs (n = 5-8) either as controls or under the influence of different physiological stimuli. The peripheral and central AVP release without physiological stimulation from the different experimental collectives was set as 100 %. The mean control values of all series were 3.2 pg AVP/ml plasma and 2.6 pg AVP/ml push-pull perfusate (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

In order to study a possible influence of noradrenergic afferents from the brainstem into the PVN on the documented antipyretic vasopressinergic pathways, we tested the febrile reactions in guinea-pigs after chronic destruction of noradrenergic afferents by 6-OHDA injection (bilateral 1 μl, 41 nmol dissolved in saline with a 1 % ascorbic acid supplement) into the PVN, or under the influence of a NA microinfusion into the PVN. The results of these experiments are summarized in Fig. 3.

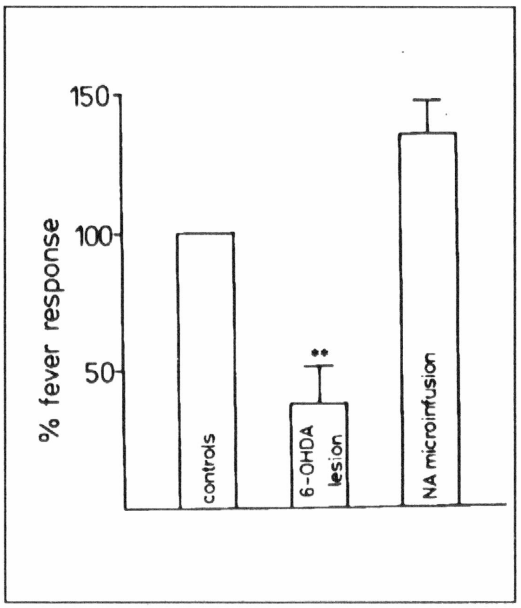

Fig. 3
Comparison of the mean fever indices from different groups of guinea-pigs (n = 6-10) under the influence of 6-OHDA lesion of noradrenergic afferents into the PVN or under the influence of noradrenaline microinfusion into the PVN. The controls received a microinjection or microinfusion of saline (** = $p < 0.01$).

In the chronic stage of the 6-OHDA lesion of noradrenergic afferents into the PVN, the fever response is significantly reduced ($p<0.01$). A microinfusion of NA into the PVN (36 μl, 21 nmol NA infused in 6 h) tends to elevate the febrile reactions of the animals. Since NA infusion already increased the body temperature before application of the pyrogen, the fever index based on the increased temperature level did not prove to be statistically significant. These results indicate an inhibitory influence of noradrenergic brain stem afferents on the antipyretic parvocellular AVP neurones projecting to the limbic septal area.

Discussion

For a substance to be legitimately categorized as an endogenous antipyretic, it should fulfill several criteria, which have recently been reviewed (Kasting 1989, Kluger 1991). From the experiments in guinea-pigs presented in this paper it becomes obvious

that AVP fulfills many of the criteria for being classified as an endogenous antipyretic:
1. The febrile response to endotoxin can be strongly reduced by infusions of AVP into a circumscribed region of the brain, the ventral part of the septal area.
2. Immunocytochemical studies reveal AVP afferents to this area from cell bodies located at least partly in the hypothalamic paraventricular and supraoptic nuclei in guinea-pigs (Dubois-Dauphin *et al.* 1989, Staiger and Nürnberger 1989) or in the bed nucleus of the stria terminalis in rats (Staiger and Wouterlood 1990). In situations characterized by reduced fever responses, these AVP pathways seem to display increased activity as shown by immunohistochemical changes (Zeisberger *et al.* 1981, Merker *et al.* 1989).
3. AVP is released in increased amounts during relevant physiological stimulations not only into the circulation, but also into the limbic septal area, where it acts as an antipyretic. This increased release of AVP is accompanied by a reduced fever response.
4. Electrical stimulation of one source of AVP neurones projecting to the septum and the PVN reduces fever, but does not suppress it completely. This indicates that additional vasopressinergic or other peptidergic afferents to the septum may be involved in fever suppression.
5. The fever reduction achieved by electrical PVN stimulation can, to a great extent, be cancelled by intraseptal application of a vasopressinergic V1 receptor antagonist. A vasopressinergic V1 specific mechanism may therefore be responsible for the antipyretic effect of AVP in the septum, which is confirmed by the existence of V1 receptors in this brain area (Gerstberger and Fahrenholz 1989). A possible additional involvement of vasopressinergic V2 receptors in the organum vasculosum laminae terminalis in endogenous antipyresis has recently been suggested (Zeisberger and Merker 1991).

From all these results, the previous suggestion to call AVP an endogenous antipyretic (Cooper *et al.* 1979) becomes more and more justified. The data shown in Fig. 1 and Fig. 2 indicate, however, that in some physiological situations there is only poor correlation between fever suppression and central and/or peripheral release of AVP. The complete suppression of fever during immobilization, for example, cannot therefore, be solely ascribed to the activation of AVP pathways to the septum. It seems rather probable that other endogenous substances are involved in antipyresis, which may modulate the release of AVP or even the action of released AVP.

In this connection, it seems to be interesting to study the noradrenergic influence from afferents originating in the lower brainstem on the antipyretic pathways. Noradrenergic brainstem afferents to thermointegrative structures of the anterior hypothalamus have been shown to play an important role in the determination of thermoregulatory thresholds (Brück and Zeisberger 1990). A microinjection of noradrenaline into the anterior hypothalamus of guinea-pigs increases heat production and body temperature (Zeisberger and Brück 1971). The thresholds for activation of heat production and heat dissipation are shifted to higher mean body temperatures by this manipulation (cf. Zeisberger and Ewen 1983) in a similar way as can be observed in the first stage of fever (Zeisberger, unpublished results). It may therefore be possible that not only the thermoregulatory thresholds but also the antipyretic vasopressinergic pathways, both being controlled by neurones in the anterior hypothalamus, can be modulated by the activity of monoaminergic brainstem afferents.

The results in Fig. 3 show that chronic destruction of noradrenergic afferents to the antipyretic neurones in the PVN reduce the fever response significantly, while microinfusion of noradrenaline into the PVN rather enhances the fever reaction. Noradrenaline released into the anterior hypothalamus facilitates the shift of thermoregulatory thresholds to a higher body temperature, which occurs during fever. The chronic destruction of the noradrenergic afferents by 6-OHDA may therefore, at least partly, inhibit the shift of thermoregulatory thresholds to a higher mean body temperature, and by this mechanism take part in antipyresis. These contemplations are, of course, still speculative, because we have not yet enough experimental evidence. Furthermore the thermointegrative structures in the anterior hypothalamus including the antipyretic neurones in the PVN receive not only noradrenergic but also serotonergic afferents which act antagonistically with regard to thermoregulation (Clark and Clark 1980, Brück and Zeisberger 1990). It becomes

obvious that both fever and endogenous antipyresis are multifunctional processes involving a number of bioactive neuropeptides and amines in their control. The interactions between these substances will have to be studied in the future.

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