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# *In vitro* Receptor Autoradiography: a Map for Exploring the Hypothalamus

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## Summary

In rabbits and guinea pigs, hypothalamic sites for prostaglandin $E_2$ ($PGE_2$) action were studied by means of *in vitro* receptor autoradiography. The density of $PGE_2$ binding sites (probably $PGE_2$ receptors) was the highest in the anterior wall of the third ventricle (A3V). This result is consistent in all mammalian species ever studied, suggesting a fundamental role of the A3V in the hypothalamic action of $PGE_2$, such as fever.

### Key words

Prostaglandin receptors – Hypothalamus – Fever

## Introduction

There are two distinct approaches to the neurophysiological study of the hypothalamus. The first one, which is very attractive, is that a number of neuromodulators have been identified there. Some of these, when injected into the hypothalamus, evoke integrated autonomic and behavioural responses. The second one, which posses the greatest difficulties, is that the neuronal organization of the hypothalamus is complex. This complexity has prevented a clear understanding of neuronal mechanisms underlying the integrated responses to these neuromodulators.

Studies of neuronal mechanisms of prostaglandin $E_2$ ($PGE_2$) induced fever are not exceptional. It is well established that $PGE_2$, when injected into the hypothalamus or into the cerebral ventricle near the hypothalamus, evokes integrated responses that induce a rise in body temperature. Heat loss decreases and/or heat production increases. If the animals are allowed to select their environmental temperature, they would choose a higher temperature. The neuronal mechanism underlying this response, however, has not been well elucidated.

*In vitro* receptor autoradiography might provide a clue to this problem by mapping the exact action sites of the neuromodulators. We have applied this technique to answer the question: where does $PGE_2$ act in the hypothalamus? Results in rats and monkeys have already been reported elsewhere (Matsumura *et al.* 1990, Watanabe *et al.* 1988). In short, the density of $PGE_2$ binding sites varies among the nuclei or the regions and is the highest in the anterior and midline portion of the preoptic area. Detailed analysis in rats further showed that this region with the highest $PGE_2$ binding corresponds to the anterior wall of the third ventricle (A3V). In the present study, we examined whether these observations are commom in other experimental animals which are often used in fever studies.

## Methods

[$^3$H]$PGE_2$ binding was determined in rabbits and guinea-pigs. They were anesthetized with thiopental (6 mg/100 g) and perfused *via* the left ventricle with cold 10 mM sodium phosphate-buffered saline (pH 7.4). Frozen serial sections of 10 $\mu$M thickness were cut in a cryostat and mounted on glass slides. The sections were incubated with 20 nM [$^3$H]$PGE_2$ in a solution containing 50 mM Tris-HCl (pH 7.4) and 0.1 M NaCl at 4 °C for 30 min. Nonspecific binding was obtained using consecutive sections by the addition of 100 M unlabelled $PGE_2$ to the incubation medium. After being dried in a desiccator, the slides were tightly juxtaposed to tritium-sensitive films in cassettes. Standard microscales for tritium concentrations were included in each cassette. After 5 weeks' exposure, the films were developed and fixed. For quantification, the optical densities of the autoradiographic images were measured using a rotating-drum scanning densitometer. According to the standard scale, the specific $PGE_2$ binding was determined.

## Results and Discussion

Detailed analysis of the distribution pattern of hypothalamic $PGE_2$ binding sites revealed a great similarity between the species yet examined (rats, guinea-pigs, rabbits and monkeys) although there are some species differences in the absolute values of the density. The highest density of $PGE_2$ binding sites was found along the A3V in all of them. Fig. 1 shows the distribution of $PGE_2$ binding sites in the A3V region of the rabbit and guinea-pig. As in rats, the binding density was especialiy high in regions close to the third ventricle or surrounding the organum vasculosum laminae terminalis (OVLT) but was low within the OVLT itself. Moderate density of $PGE_2$ binding sites was also found in the posterior part of the hypothalamus, being similar to that in rats.

The great similarity of $PGE_2$ binding sites betweeen the species suggests its fundamental role in the mammalian central nervous system. The binding sites identified by an autoradiographic technique often correspond to functional receptors to the neurotransmitters or neuromodulators. If this is also the case in our $PGE_2$ binding study, the A3V seems to play an important role in the action of $PGE_2$ in the hypothalamus. Based on our autoradiographic data, we propose a working hypothesis that the A3V is important in the febrile action of $PGE_2$ and design detailed experiments to verify this hypothesis.

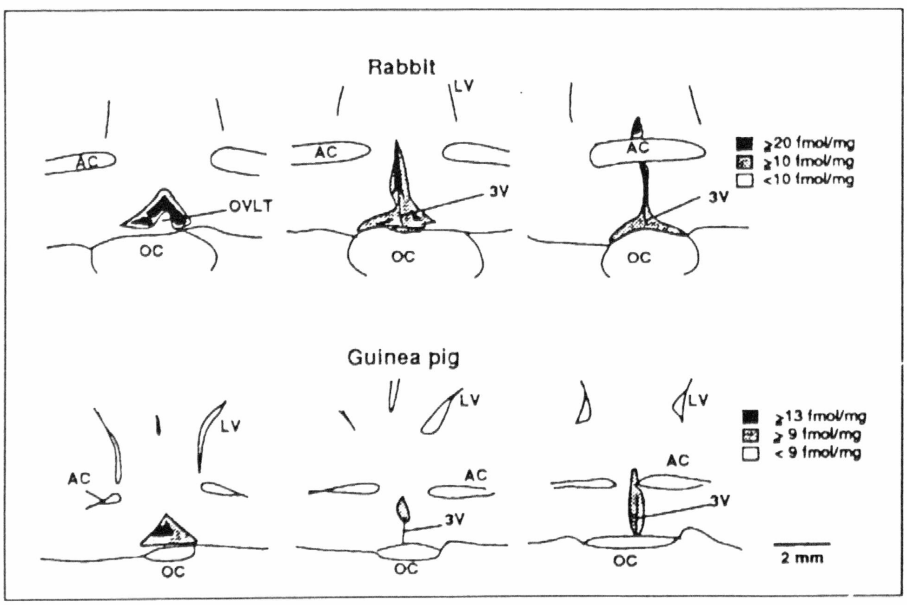


**Fig. 1**

$PGE_2$ binding sites in the coronal sections of rabbit (upper) and guinea-pig (lower) preoptic-hypothalamic region containing three different levels of A3V from rostral (left) to caudal (right). AC: anterior commissure, OVLT: organum vascolosum laminae terminalis, OC: optic chiasma, LV: lateral ventricle, 3V: third ventricle.

## References

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