Interaction between the effects of centrally administered arecoline and leucocyte pyrogen on the activity of posterior hypothalamic neurons in the rabbit

V. N. Gourine, V. V. Tsaryuk and A. G. Fitton

Department of Normal Physiology, Minsk State Medical Institute, Minsk (USSR), 8 June 1984

Summary. In experiments with urethane-anesthetized rabbits, the alteration in the activity of posterior hypothalamic neurons resulting from intracerebroventricular injection of leucocyte pyrogen was attentuated by subsequent administration of arecoline. Atropine failed to alter the neuronal response to leucocyte pyrogen but abolished the effect of arecoline. The neuronal response to arecoline was reversed in the absence of leucocyte pyrogen.
Key words. Leucocyte pyrogen; arecoline; posterior hypothalamus; single-unit activity.

It is generally accepted that endogenous pyrogen acts primarily on target structures within the preoptic/anterior hypothalamic area (PO/AH) to produce fever. Rosendorff and Mooney[1] have demonstrated that microinjection of leucocyte pyrogen into the PO/AH induces fever in the rabbit; in addition, they have described a secondary endogenous pyrogen-sensitive site in the midbrain. Systemic administration of endogenous pyrogen has been shown to modify the activity of thermosensitive neurons in the PO/AH[2,3], anterior hypothalamus[4], midbrain and brainstem[5].

A number of neurotransmitters have been suggested as being involved in determining the activity of those hypothalamic neurons which participate in thermoregulatory processes under normal physiological conditions and during fever[6]. In a wide range of species, acetylcholine has been shown to lower body temperature following central administration. Cholinomimetics have similarly been reported to display such an action in the case of fever induced by exogenous pyrogen[7].

It has been suggested that certain neurons of the posterior hypothalamus play an integrative role in thermoregulatory processes[8]. In accordance with the neuronal model of the hypothalamic thermoregulatory center proposed by Myers[9], the thermoregulatory pathways of the posterior hypothalamus are composed of cholinergic neurons. We therefore decided to study the effects of the cholinomimetic arecoline and the cholinolytic atropine on the activity of posterior hypothalamic neurons following central administration of leucocyte pyrogen.

Methods. Experiments were performed on 12 New Zealand rabbits weighing 2.8–3.5 kg which were anesthetized with urethane (1.3 g/kg i.p.) and maintained under thermoneutral conditions (20–24 °C). The animal's head was positioned in a stereotaxic frame, the scalp was opened and a section of parietal bone 3 mm in diameter was removed to expose the dorsal surface of the cortex. A tungsten micro-electrode was stereotaxically positioned to record the extracellular impulse activity of single posterior hypothalamic neurons (co-ordinates $P_1L_1G_{14\text{-}15}$ according to the atlas of Sawyer, Everett and Green[11]). Unit responses were amplified using a UBP-02 amplifier and displayed visually on an oscilloscope screen (C1-19B) prior to subsequent transformation into standard square-wave impulses of 2.5 msec duration using an amplitude discriminator[12], a procedure which allowed the unit response frequency to be recorded on a H-327 servoscribe. Simultaneously, the responses were processed using an AMG-1 impulse analyzer which calculated the mean impulse frequency over 4 sec time intervals.

Aqueous drug solution were injected via implanted cannulae into the lateral cerebral ventricles in volumes not exceeding 30 µl. The drugs employed were arecoline, atropine sulphate and leucocyte pyrogen (prepared from the endotoxin *S. typhi* according to the technique described previously by Cranston, Hellon and Townsend[13]. Drug effects were evaluated by comparing the mean impulse frequency prior to and following injection of the various solutions. Changes in impulse frequency in excess of 25% of the basal level were regarded as being significant, exceeding by a factor of 2.5 the threshold level for fluctuations in impulse frequency necessary for synaptic transmission of information[14]. Non-parametric statistical analyses based on the sign test and the method of Fisher were used to quantify the significance of these changes[15]. In accordance with morphological findings, the neurons under investigation were located in the dorso-medial nuclei and the posterior region of the hypothalamus.

Results. Investigation of the responses of 22 posterior hypothalamic neurons indicated that intracerebroventricular administration of leucocyte pyrogen (30 µl/rabbit) significantly frequently ($p < 0.05$) enhanced neuronal activity, producing an increase in the firing rates of 13 neurons and a decrease in those of 3 neurons, while failing to alter the intrinsic activity of 6 neurons. Investigation of the effects of arecoline and atropine on neuronal activity was confined to those neurons previously shown to respond to leucocyte pyrogen. It was found, in all instances, that intracerebroventricular administration of arecoline (50 µg) 7–12 min after leucocyte pyrogen produced a neuronal response opposite to that of leucocyte pyrogen. Hence, all 8 neurons activated by leucocyte pyrogen reduced their firing rates in response to subsequent intracerebroventricular administration of arecoline; the frequency of this response was significant ($p < 0.01$). One neuron whose activity was inhibited by leucocyte pyrogen responded to arecoline with an increase in its firing rate. The effects of intracerebroventricular administration of atropine (100 µg) were determined on 9 neurons previously shown to be activated by leucocyte pyrogen. It was found that intracerebroventricular administration of atropine 7–12 min after leucocyte pyrogen did not significantly alter the neuronal response to leucocyte pyrogen. Intracerebroventricular administration of the aqueous vehicle (30 µl) had no significant effect on neuronal activity. The effects of arecoline on neuronal activity were reduced or completely abolished by atropine (100 µg) administered intracerebroventricularly 5 min prior to the cholinomimetic. A typical neuronal response to leucocyte pyrogen, arecoline and atropine is depicted in figure 1.

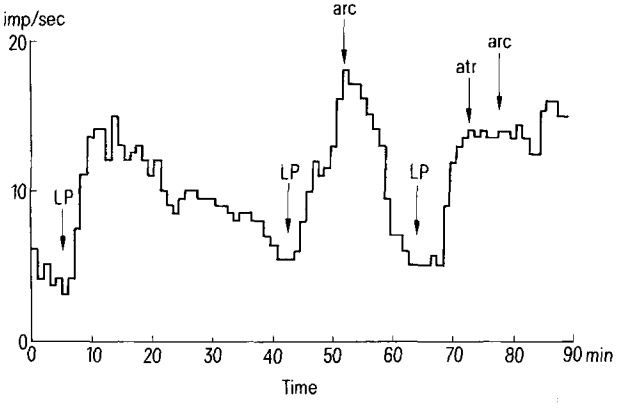

Figure 1. The impulse activity of a single posterior hypothalamic neuron in response to a) leucocyte pyrogen (30 µl i.c.v.) and b) arecoline (50 µg i.c.v.) and atropine (100 µg i.c.v.). during the leucocyte pyrogen-induced increase in neuronal activity. LP: leucocyte pyrogen; arc: arecoline; atr: atropine.

In a series of control experiments the effects of arecoline on the activity of posterior hypothalamic neurons were determined in the absence of leucocyte pyrogen. As before, the study was confined to those neurons previously shown to respond to leucocyte pyrogen. In the absence of leucocyte pyrogen intracerebroventricular administration of arecoline (50 µg) enhanced neuronal activity in a significant proportion of cases ($p < 0.01$), producing an increase in the firing rates of 8 neurons and a decrease in those of 0 neurons while failing to alter the activity of 1 neuron. When administration of arecoline was repeated 7–12 min after intracerebroventricular administration of leucocyte pyrogen (30 µl) the opposite neuronal response was observed; in this case the leucocyte pyrogen-induced increase in neuronal activity was attenuated by the cholinomimetic in all 10 neurons investigated ($p < 0.01$). Statistical analysis (Fisher's method) of the experimental and control data indicates that there was a significant qualitative difference ($p < 0.025$) between the neuronal responses to arecoline in the absence and presence of leucocyte pyrogen. A typical neuronal response to arecoline under these conditions is depicted in figure 2.

Discussion. The results obtained indicate that leucocyte pyrogen and arecoline act centrally in dissimiliar manners to influence the activity of posterior hypothalamic neurons. It would appear that their observed interaction at the level of the posterior hypothalamus is of a physiological, rather than a pharmacological, nature. It is generally accepted that leucocyte pyrogen fever is initiated through an action at the PO/AH. The fact that the ability to develop fever is only fully realized when the posterior hypothalamus is intact[16] does, however, implicate posterior hypothalamic pathways in the subsequent development of the febrile response. The site of action of arecoline in the above-mentioned interaction is less clear. The fact that the posterior hypothalamus contains a large number of cholinergic neurons[17], coupled with the proposition[10] that the thermoregulatory pathways of the posterior hypothalamus are cholinergic, might suggest that arecoline is acting directly on posterior hypothalamic neurons. However, the possibility that the effect of arecoline is mediated through an action on the anterior hypothalamus cannot be discounted. It has been shown[18] that anterior hypothalamic cholinergic pathways exert an inhibitory influence on the activity of the dorso-medial nucleus (shivering center) in the rabbit: whereas injection of arecoline into the anterior hypothalamus inhibited neuronal activity in the dorso-medial nucleus, injection into posterior hypothalamic sites was without effect. Moreover, the diversity of the neuronal response to arecoline observed in the absence and presence of leucocyte pyrogen might suggest that the action of the cholinomimetic on posterior hypothalamus neurons is not a direct one.

There is substantial evidence to indicate that the posterior hypothalamus contains neurons which play an integrative role in thermoregulatory processes[9,19,20]. The results of the present investigation indicate that, on the basis of its M-cholinomimetic properties, arecoline is able to counteract the influence of leucocyte pyrogen on certain posterior hypothalamic neurons. This raises the possibility that the observed changes in the firing rates of these neurons are reflections of alterations in the activity of neuronal groups sending efferent signals to thermoregulatory effectors. It is interesting to note that a similar antagonistic effect has been described between the action of leucocyte pyrogen and the antipyretic Sulpyrine on the activity of individual thermosensitive neurons of the rabbit medulla[21]. In the present series of experiments leucocyte pyrogen failed to evoke fever, since the study was performed with anesthetized animals. However, we have previously shown that intracerebroventricular administration of leucocyte pyrogen (50 µl) to conscious rabbits results in the development of a significant febrile response[22]. In addition, we have demonstrated that arecoline causes vasodilatation and a significant reduction in body temperature during leucocyte pyrogen fever in the rabbit[8]. In view of this, the present findings allow us to propose that those posterior hypothalamic neurons whose activity is enhanced by leucocyte pyrogen and subsequently reduced by arecoline participate in regulatory heat gain mechanisms.

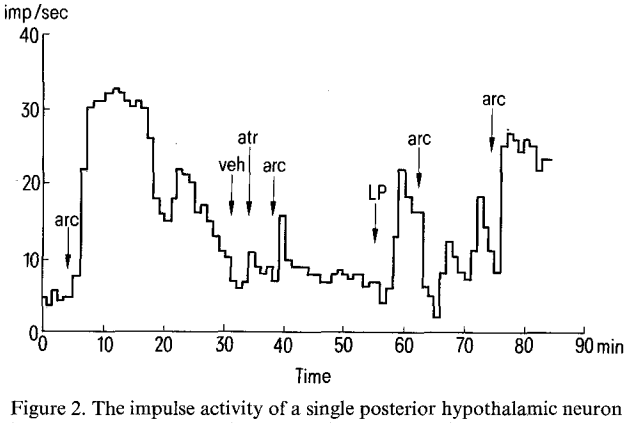

Figure 2. The impulse activity of a single posterior hypothalamic neuron in response to arecoline (50 µg i.c.v.) prior to and following administration of leucocyte pyrogen (30 µl i.c.v.). The initial response to arecoline is restored once the effect of leucocyte pyrogen has subsided. The response to arecoline is blocked by atropine (100 µg i.c.v.). LP: leucocyte pyrogen; arc: arecoline; atr: atropine; veh: aqueous vehicle.

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