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Effects of pyrogen and antipyretic on thermoregulatory effector functions in rabbits*

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Abstract: Effect of sulpyrine antipyretic on thermoregulatory effector functions was studied on conscious febrile rabbits. In LPS-pyrogen induced febrile rabbits, receiving sulpyrine by intravenous injection, there occurred a rapid fall in hypothalamic temperature differed significantly from the fall in retal temperature. And this dissociation of temperatiures between the PO/AH and rectum was succeeded not only by various evaprative heat loss such as thermal panting and peripheral cutaneous vasodilation, but metabolic heat production. This opposing, crossed thermoregulatory responses might be induced by the additive interaction between hypothalamic and extrahypothalamic temperature signals both being further modified in afferent cold-warm pathways, by local hypothalamic temperature as well as by chemical action between pyretic and antipyrtic in CNS.

Key words: LPS-pyrogen, sulpyrine, temperature regulation, crossed thermoregulatory responses

PREFACE

It is particularly important to determine the possible effect of hypothalamic blood flow changes on fluctuations in hypothalamic temperature. In this problem, we recently reported that during LPS pyrogen-induced fever in rabbits, the blood flow was increased or decreased with similar shifts of biphasic character in hypothalamic temperature (Inomoto *et al.*, 1979 -a, -b Kosaka *et al.*, 1982 -a, -b). In a series of the same experiments,

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after intravenous injection of antipyretic (sulpyrine), both evaporative heat loss and heat production (increase of oxygen consumption) were simultaneous by induced in febrile rabbits. Therefore, the immediate object of the present investigation is to interpret the functional discrepancy of interaction which occurs between pyretic and antipyretic substances administered intravenously to the rabbit in driving thermoregulatory responses.

METHOD

Estimation of oxygen consumption was performed as follows: the trachea canula was connected with a Benedict Roth's respirometer (13.5 liters, Bell factor 41.4 ml/mm) by means of rubber tubing, as shown in Fig. 1. Thus a closed circuit was formed between the animal and respirometer in which respiratory gases were enough perfused for contacting soda-lime in the cylinder by using two perfusions air pumps. Almost no artificial pressure exists in the respiratory passage of the rabbit. Gaseous temperature in the respirometer was kept constant by faint cooling the gases in the closed circuit system (Kosaka *et al.*, 1975). Oxygen consumption estimated in V_{atps} in every 15 minutes periods was converted into V_{stpd} after referring to the coefficient table of Peters. The Peter's coefficient was calculated from the fact that soda-lime absorbs 20% of water vapor in the closed circuit system, therefore, V_{stpd} was reduced from the ambient temperature, pressure and 80% of water vapor saturated in the present investigation. Temperatures of rectum (T_r), hypothalams (T_{hy}) and ear skin (T_e) were continuously measured with thermocouples. The respiratory frequency (RR) was detected from the resistance changes of strain gauge. Blood flow of the common carotid artery (F.C.C.A.) and Heart rate (HR) were measured with an electromagnetic flow meter and the modified system (Nihon Koden Co.), respectively. Test animals (albino rabbit) were administed 1−3 μg/kg LPS pyrogen (Lipopolysaccharide, from E. coli B6, Sigma), distilled in 2 ml physiological saline solution, through the retroauricular vein. Antipyretic (15−20 mg/kg, sulpyrine) was given intravenously during the first and second phase of LPS-pyrogen induced fever. The experiment was carried out in an environmental control chamber at constant temperature and humidity (28°C, 60%).

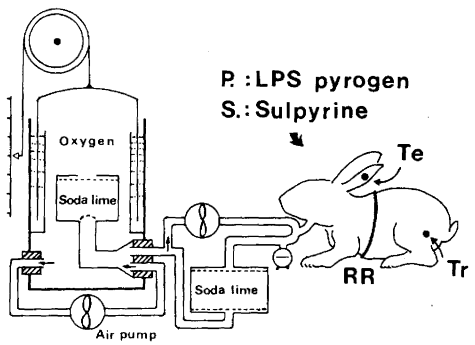

Fig 1. An experimental survey and a measurement of oxygen consumption by a closed circuit system with a Benedict Roth's respirometer.

RESULTS

(1) A typical finding is demonstrated in Fig 2 which shows that the intravenous injection of LPS-pyrogen caused not only a rapid rise in hypothalamic and rectal temperatures of 1.5 −1.0°C but also themoregulatory heat conservation and heat production such as ear skin vasoconstriction (Te), bradypnea (RR), increase in heart rate (HR), as well as in blood flow of common carotid artery(F.C.C.A.), during first 60 minutes. In this rabbit receiving sulpyrine by intravenous injection at an arrow point in the Fig 2 (S), there was a rapid fall in hypothalamic temperature which was significantly different from the zall in rectal temperature. And this dissociation between hypothalamic and rectal temperature was succeded by various thermoregulatory heat loss responses such as peripheral vasodilation (Te), thermal panting (RR), decrease in heart rate (HR), as well as in F.C.C.A. and Tr.

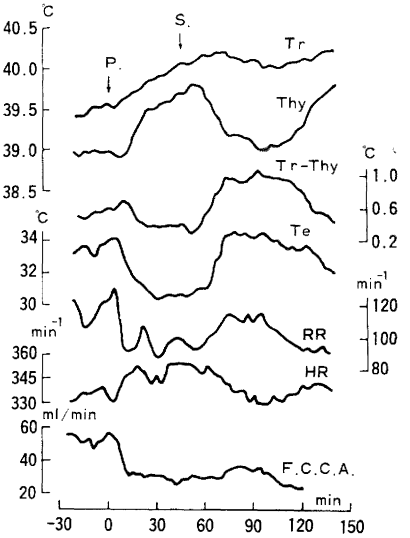

Fig 2. Temperatures of rectum (Tr), hypothalamus (Thy) and ear skin (Te). Respiratory rate (RR), heat rate (HR), and blood flow of common carotid artery (F.C.C.A). At the first arrow, intravenous injection of LPS-pyrogen (P.): at the second arrow of sulpyrine (S.) during the first phase of LPS-pyrogen induced fever.

(2) In a series of experiments in LPS pyrogen induced febrile rabbits, both evaporative heat loss and heat production (increase of oxygen consumption) were simultaneously evoked by intravenous injection of antipyretic sulpirine, as shown in Fig. 3 and Fig. 4. Following the start of febrile reaction, the rabbits assumed a compact posture and there ear skin vessels remained constricted. Cold shivering was seen only occasionally in these lightly restrained rabbits. Rectal temperatures rose by 0.5°C during the first period (Fig. 3) as well as by 1.5°C during the second period (Fig. 4) of LPS- pyrogen fever at 70 and 180 minutes after administration respectively. In these febrile rabbits, sulpyrine administered by intravenous injection caused thermoregulatory heat dissipation such as cutaneous vasodilation (Te), fall in rectal temperature (Tr) and thermal panting (RR) as demonstrated in Fig. 3 and Fig. 4. And there heat loss responses were induced by a marked dissociation between Thy and Tr as mentioned in Fig. 2.

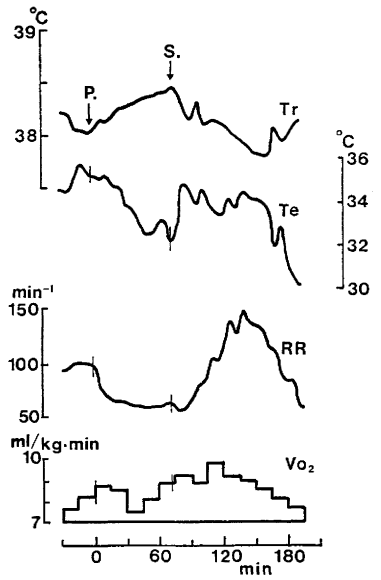

Fig 3. Temperatures of rectum (Tr) and ear skin (Te). Respiratory rate (RR) and oxygen consumption (Vo_2). At the first arrow, intravenous injection of LPS-pyrogen (P.): at the second arrow of sulpyrine (S.) during the first phase of pyrogen fever.

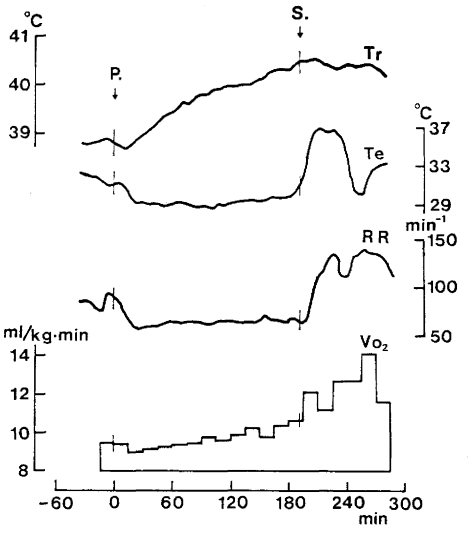

Fig 4. Temperatures of rectum (Tr) and ear skin (Te). Respiratory rate (RR) and oxygen consumption (Vo_2). At the first arrow, intravenous injection of LPS-pyrogen (P.): at the second arrow of sulpyrine (S.) during the second phase of pyrogen fever.

(3) As show at bottom of Fig. 3 and Fig. 4, however, significant increase in oxygen consumption concerned with heat production were simultaneously evoked during antipyretic period by intravenous sulpyrine. In order to explain the functional discrepancy of interaction between pyretic and antipyretic substances used in the present experiment, effect of sulpyrine on thermoregulatory heat production was examined in control rabbits (with no LPS pyrogen) fixed lightly in all fours. None of the intravenous doses of sulpyrine caused any change in rectal temperature as well as in oxygen consumption in all experiments in control rabbits, and the results from the typical experiment is shown in Fig. 5.

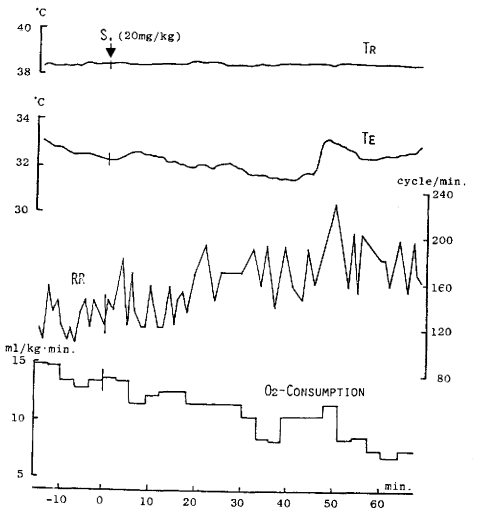

Fig 5. Temperatures of rectum (Tr) and ear skin (Te). Respiratory rate (RR) and oxygen consumption (O_2-consumption). At the arrow, intravenous injection of sulpyrine (S.) in the afebrile state of the contral rabbit.

DISCUSSION

It appears that there are at least possibly two mechanisms by which antipyretics (salicylates and sulpyrine) produce their antipyretic effects. Firstly, they appear to prevent the formation or release of endogenous pyrogen (EP) by leucocytes (especially, lymphocytes and macrophages) in the rabbit (Rawlins, 1973). Secondly, it is likely that the antipyretic effects of salicylate or sulpyrine depends on its concentration at an intracranial site, and they competitively antagonise the effects of EP within the preoptic hypothalamus (PO/AH) and midbrain, by the following manner that the suppression of firing rate in thermosensitive neurons in the PO/AH or midbrain by the administration of pyrogen is reduced by the injection of the antipyretics (salicylates and sulpyrine) intravenously (Wit & Wang 1968). As a working hypothesis it is suggested that antipyretic exerts its antipyretic action by interfering with the process involved in the genesis of fever (Cranston *et al.*, 1970). This hypothesis is based on the fact that in a febrile rabbits and in normal volunteers salcylate and sulpyrine have no influence upon either temperature or thermoregulatory reflex as demonstrated in Fig. 5 in the present experiment. Therefore, as shown in Fig. 7, whether antipyretic interferes in some manner with possible interaction between endogenous pyrogen, PO/AH neurones and peripheral nerve receptors or whether the increased effect of antipyretic on firing rate in PO/AH warm neurones is due to a direct effect on the neurons, remains uncertain. Thermoregulatory functional discrepancy of interaction between LPS-pyrogen and sulpyrine antipyretic, that is after administration of sulpyrine, both evaporative heat loss and metabolic

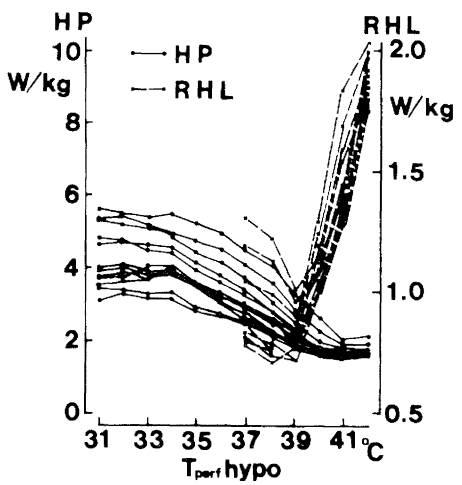

Fig 6. Overlapping of threshold temperatures for heat production (HP) and respiratory evaporative heat loss (RHL) as functions of hypothalamic perfusion temperature (Tperf). The schema modified and cited from Jessen (1977).

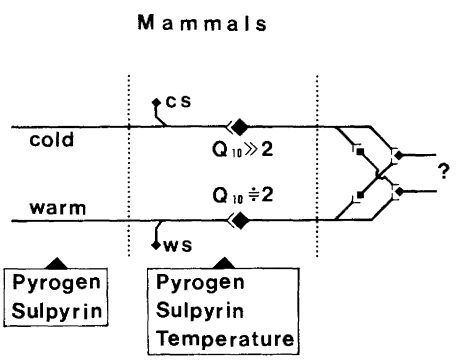

Fig 7. Diagrammatic respresentation of how temperature dependence of afferent intrahypothalamic neural pathways may be arranged in mammals to explain the effects of hypothalamic cooling as well as of pyretic and antipyretic on thermoregulation. Diagram derived and cited from Lin and Simon (1982).

heat production were simultaneously observed in febrile rabbits as shown in Fig. 3 and Fig. 4 in the present results. A similar finding on the overlapping of threshold temperatures of hypothalamus for heat production and respiratory evaporative heat loss was demonstrated in the goat (Jessen, 1977). And Jessen reported that as function of hypothalamus perfusion temperature in a zone of approximately 2°C around normal body core temperature, both thermoregulatory heat production and heat loss functions were simultaneously altered by spinal temperature and by hypothalamic one, as demonstrated in Fig. 6. Furthermore the similar phenomenon was reported in the hypothermic dogs which were subjected to strong thermal panting by continuous heating of the PO/AH region and which shivered with panting just immediately after discontinuing the heating of the PO/AH (Nakayama, 1984). In the present experiment, however, after intravenous injection of sulpyrine, there occurred a rapid fall in PO/AH temperature which was significantly different from the fall in rectal temperature. And this marked fall in PO/AH temperature caused the dissociation of temperatures between the hypothalamic and extrahypothalamic thermosensitive tissues. From the aspect related such in brain cooling of the fall in PO/AH temperature, the experimental results in birds, lowering hypothalamic temperature induces cutaneous vasodilation and suppression of shivering, contrary to mammals, is acceptable for explaining the present crossed results of increase in oxygen consumption during thermal panting and cutaneous vasodilation as shown in Fig. 3 and Fig. 4. Namely, in studies on ducks, this paradoxical effects of PO/AH cooling on avian thermoregulation could be characterized by a decrease of the threshold body temperatures tor effector activation and of the sensitivities with which the effectors respond to changes of body temperature (Simon-Opperman & Simon, 1980). And these effects were attributed to a non-specific temperature dependence of the hypothalamic integrative network and to the non-existence of cold receptors in the hypothalamus (Inomoto, 1982). Based on neurophysiological studies on ducks, the changes of thresholds and sensitivities were explained by the hypothesis that the activities of hypothalamic neurones conveying extrahypothalamic cold signals are depressed more by hypothalamic cooling than the activities of those neurones which convey the warm signals (Eissel & Simon, 1980), and recently, a higher Q_{10} of cold signal than warm signal pathway in the duck's hypothalamus was directly demonstrated by using electrophysiological method (Lin and Simon, 1982). If we accepts that birds and mammals may have inherited the same neural arrangement in the CNS thermoregulatory network from their ancesters, the opposing effects hypothalamic cooling on avian and mammalian effector activities can be explained by additionally assuming the existence of primary thermoreceptors in the mammalian hypothalamus as shown in Fig. 7, which was derived from Lin & Simon (1982). According to the model in this Fig. 7, the opposing, crossed thermoregulatory responses in the preset results might be induced by an additive interaction between hypothalamic and extrahypothalamic temperature signals both being further modified in their afferent

pathways by local hypothalamic temperature as well as by chemical action between pyretic and antipyretic in CNS.

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ウサギの発熱・解熱過程における体温調節反応

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ウサギの LPS-pyrogen による発熱曲線の解析や sulpyrine 解熱過程における体温調節反応について次の結果を得た。a)正常ウサギに sulpyrine 静注しても，直腸温，呼吸数，耳介皮膚温，酸素消費量などの体温調節反応に有意な変化を認めない．b)正常ウサギの直腸温と視床下部温はほぼ平行推移するが LPS-pyrogen 静注による発熱初期や sulpyrine 解熱初期には直腸温と視床下部温の間には顕著な解離があり，特に視床下部温低下および脳温・脳血流・脳代謝との相関について検討する必要がある．c)sulpyrine 解熱過程にみる熱放散反応（耳介皮膚温上昇および panting の出現）と同時に誘起する熱産生反応（酸素消費量の増加）の所見を下記の如く解釈している．即ち，ヤギの視床下部温低下（39°C→37°C）の過程で熱産生反応と呼吸性熱放散が同時に誘発されるとの報告の如く，本研究における LPS-pyrogen 発熱ウサギの sulpyrine 解熱時に誘起された酸素消費量の増加は，pyrogen と sulpyrine の中枢性・末梢性・温・冷ニューロンへの相乗作用及び解熱過程にみる視床下部温低下が相加作用した結果，体温調節反応系に誤差信号が発生したと推測している．この解釈は視床下部における信号伝達の局所温依存性の概念に通じるものと考えている．

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