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THE EFFECTS OF INTRACEREBROVENTRICULAR CYCLOHEXIMIDE ON PROTEIN SYNTHESIS AND FEVER IN RABBITS

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Cycloheximide (40 μg) intracerebroventricularly (i.c.v.) had no effect on thermoregulation against cold, but reduced fever due to an i.c.v. injection of leucocyte pyrogen (LP) by 25%, and reduced incorporation of radioactive leucine into hypothalamic protein by 94%.

Introduction Fever is now generally believed to be mediated by endogenous pyrogen (EP), a protein which is produced by cells of the reticuloendothelial system and which acts on the preoptic area of the anterior hypothalamus to raise body temperature. Various substances have been suggested as being involved in the EP-fever pathway (see review by Cranston, 1979) but the various steps are still largely unknown.

Siegert, Philipp-Dormston, Radsak & Menzel (1976) showed that the febrile response to intravenous (i.v.) EP in the rabbit could be inhibited by i.v. injection of the protein synthesis inhibitor, cycloheximide. The response to intracerebroventricular (i.c.v.) EP was also inhibited by i.v. cycloheximide (Hellon, Cranston, Townsend, Mitchell, Dawson & Duff, 1979), indicating that cycloheximide was not acting by preventing access of EP to the ventricles, but that perhaps one of the steps in the febrile pathway involved protein synthesis.

Doubt has been cast upon this interpretation by the work of Barney, Katovich & Fregly (1979), who showed that systemic treatment of rats with cycloheximide interfered with thermogenesis and made them unable to maintain a stable core temperature when exposed to cold. Stitt (1980) showed the same effect in rabbits, and also demonstrated that cycloheximide had no effect on fever in a hot environment, where the febrile response is due to a reduction in heat loss rather than an increase in heat production.

Since then, we have used another protein synthesis inhibitor, anisomycin: given i.c.v., it reduced fevers due to i.v. or i.c.v. leucocyte pyrogen (LP), and had no effect on normal thermoregulation (Cranston, Hellon & Townsend, 1980).

We therefore decided to look at the effects of cycloheximide on fever, thermoregulation against cold and protein synthesis, when the drug was given into the lateral ventricles.

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Methods Rabbits were fitted with stereotaxic headplates (Monnier & Gangloff, 1961) under general anaesthesia, to allow injection into the lateral ventricles, and one week allowed to elapse before they were used as experimental subjects. Experiments were carried out in a temperature-controlled room at 21°C (except for cold exposure); rabbits were restrained in conventional stocks and their body temperatures measured by rectal thermocouples, printing every 10 min on a digital microvoltmeter. Before each experiment, the sterility of the injection system was checked by establishing that no change in rectal temperature followed within an hour of the i.c.v. injection of 0.1 ml artificial cerebrospinal fluid (CSF) (Cameron & Semple, 1968). Except for the ^{14}C incorporation experiments, each rabbit received injections of cycloheximide dissolved in 40 μl CSF, or CSF, in random order, acting as its own control. At least 48 h separated the injections. The method by which LP was made has been described previously (Cranston *et al.*, 1980).

The effect of i.c.v. cycloheximide on thermoregulation against cold was investigated by exposing 10 rabbits to a cold environmental temperature of 8°C. After 90 min, they were given either 40 μg cycloheximide i.c.v. or 40 μl CSF i.c.v.; their rectal temperatures were followed for 3 h.

Ten rabbits each received an i.c.v. injection of 40 μg cycloheximide or 40 μl CSF, 10 min before an i.c.v. injection of 10 μl LP. Experiments were performed at 21°C, and rectal temperatures monitored for 3 h.

The effect of i.c.v. cycloheximide on hypothalamic protein synthesis was examined by giving 4 rabbits 40 μg cycloheximide i.c.v., and 4 rabbits 40 μl CSF i.c.v., 10 min before all received 2.5 μCi ^{14}C-labelled leucine i.c.v.; 15 min later the rabbits were killed, the hypothalamic area removed, and the protein extracted by a modification (Cranston *et al.*, 1980) of the method of Grahame-Smith (1972). The radioactivity incorporated into hypothalamic protein was then measured in a scintillation counter, and expressed as disintegrations per minute (d/min) per mg protein.

Results Intracerebroventricular injection of 40 μg cycloheximide had no significant effect on thermoregulation against cold (Figure 1a) but reduced the

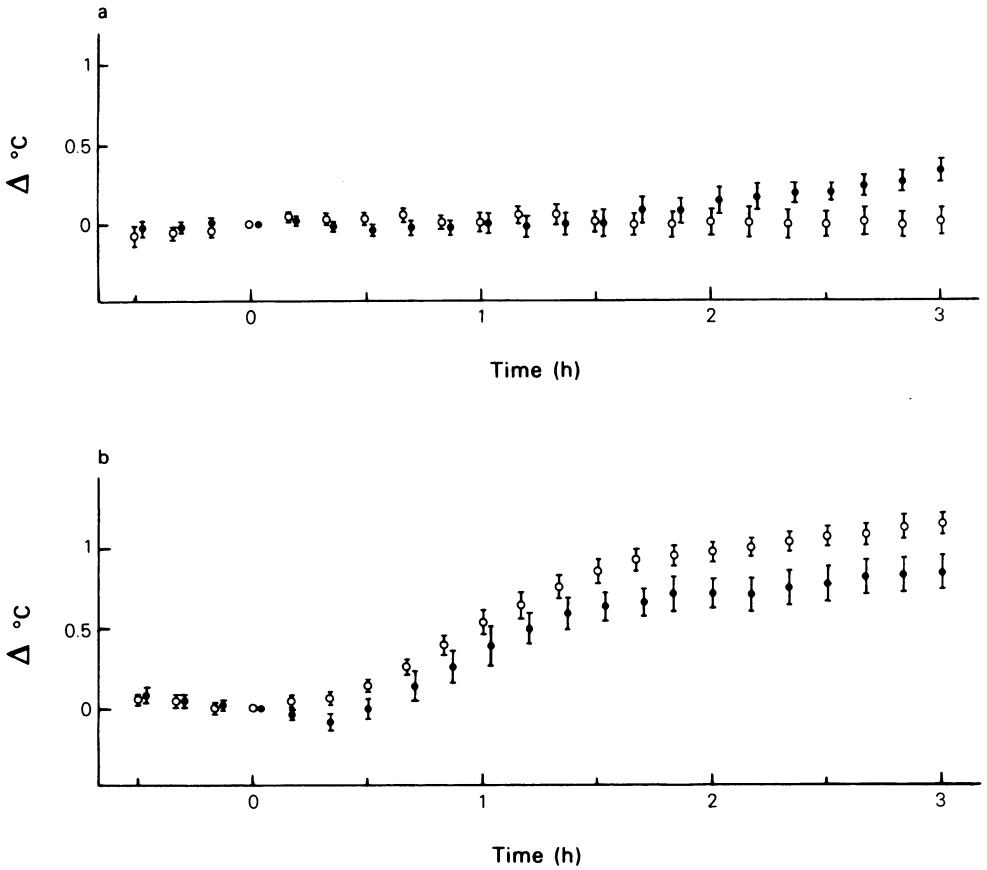

Figure 1 (a) Effect of i.c.v. injection of 40 μg cycloheximide (●) or 40 μl CSF (○) on rectal temperature of 10 rabbits exposed to an environmental temperature of 8°C. Ordinate scale: change in rectal temperature; abscissa scale: time after injection. Each point represents mean, vertical lines show s.e. mean. (b) Effect of i.c.v. injection of 40 μg cycloheximide (●) or 40 μl CSF (○) at −10 min, upon the fever resulting from injection of 10 μl leucocyte pyrogen into the lateral ventricles of 10 rabbits at time zero. Ordinate scale: change in rectal temperature; abscissa scale: time after injection of LP. Environmental temperature = 21°C.

febrile response to i.c.v. LP (Figure 1b) by approximately 25% (measured as either maximum height of fever reached in 90 min ($t=2.87$; $P<0.02$) or in 3 h ($t=2.59$; $P<0.05$), or as area under the 3 h temperature curve ($t=2.71$; $P<0.025$). Incorporation of radioactive leucine into hypothalamic protein was reduced by 94%, from 1864±352 d min^{-1} mg^{-1} to 115±9.6 d min^{-1} mg^{-1}, by 40 μg cycloheximide ($t=4.96$; $P<0.005$).

Discussion Although the effect on fever of 5 mg/kg cycloheximide given intravenously is suspect, the results presented here indicate that there is a true reduction of the febrile response when cycloheximide is given directly into the cerebral ventricles at a dose of 40 μg. This effect is not due to either impairment of thermoregulation against cold or prevention of entry of LP into the brain.

The fact that two different inhibitors of protein synthesis, anisomycin and cycloheximide, attenuate the febrile response to LP given i.c.v., without impairing the animals' ability to maintain core temperature in the cold, favours the view that the production of fever requires central protein synthesis. It argues against pharmacological actions of the compounds, independent of their effects on protein synthesis.

V.N.G. is a W. H. O. Fellow.

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