Dimethyl sulphoxide, a pyrogen potentiating agent

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Dimethyl sulphoxide (DMSO), a dipolar organic solvent of low toxicity *in vivo* (David, 1972; Rubin, 1975; Brobyn, 1975) is widely known for its ability to facilitate the transfer of drug molecules across biological membranes (David, 1972; Wood & Wood, 1975). DMSO has also been shown to elicit primary pharmacological effects (Jacob, Bischel & Herschler, 1964; David 1972) which include anti-inflammatory and analgesic activities. Little is known, however, about an antipyretic effect of DMSO. Dermal application to the back of the neck of the rat with a persistent fever did not show an antipyretic effect (C.Z. Technical Bulletin, 1963). On the other hand Jacob in a personal communication reports an antipyretic effect of parenterally administered DMSO in rodents and dogs (David, 1972). Because of these different findings and the lack of other data in the literature we have examined the effect of DMSO on fever induced with exogenous pyrogens. Since endogeneous pyrogen production by blood or tissue leucocytes has been clearly implicated as the cause of experimental fever due to bacterial pyrogens, viruses and antigen-antibody interactions (Atkins & Snell, 1974), the effect of DMSO on fever induced with leucocytic pyrogen (LP) was also considered. DMSO (BDH chemicals Ltd. Poole, England) was injected intravenously as a 25% v/v solution in pyrogen-free saline at a dose rate of 2 ml kg^{-1} 15 min before the administration of the pyrogen. In most cases a second dose was given 45 min after the administration of the pyrogen. The exogenous pyrogens used were sodium nucleate from yeast (E. Merck, Darmstadt, West-Germany) and purified lipopolysaccharides (LPS) from *Salmonella typhimurium* or *Escherichia coli* (Difco Laboratories, Detroit, Mich. U.S.A.). The dose of LP was 2 or 4 ml kg^{-1} (equivalent to 4×10^7 or 8×10^7 leucocytes kg^{-1} respectively). The dose of plasma containing endogenous pyrogen (EP) was 15 ml kg^{-1} administered intravenously in rabbits made tolerant to endotoxin by three daily injections of LPS *E. coli* 1 μg kg^{-1}. Methods for maintaining

Table 1. *The effect of dimethyl sulphoxide (DMSO) on LPS, sodium nucleate or LP induced fever in rabbits*

Pyrogen	Dose kg^{-1} b.w.	Fever index with 25% v/v DMSO (F.I.5) mean ± s.e.	2 ml (i.v.) kg^{-1} b.w.	Fever index mean ± s.e.	n*
Control (saline)	2 ml	5 ± 2·4	—	—	5
—	—	—	−15′ and +45′	3 ± 1·4	5
LPS *S. typhimurium*	0·001 μg	10 ± 3·2	" "	20 ± 4·8	5
	0·01 μg	20 ± 3·6	" "	40 ±10·0	5
	0·025 μg	24 ± 4·4	" "	46 ± 7·2	5
	0·05 μg	26 ± 8·7	" "	55 ± 4·7	5
	0·1 μg	44 ± 6·5	" "	58 ±10·6	10
	0·2 μg	57 ± 8·1	" "	72 ± 7·5	5
LPS *E. coli*	0·01 μg	31 ± 3·8	" "	56 ± 5·7	6
Sodium nucleate	40 mg	65 ± 10	— —		3
	1 mg	31 ± 5·6	" "	62 ± 13	3
	1 mg		−15′	57 ± 7·4	3
LP†	4×10^7	9·8 ± 2·8‡	"	6·2 ± 1·0	4
	8×10^7	14·6± 2·0	"	13·7 ± 2·0	4

* n = Number of rabbits used.

† The dose of LP (leukocytic pyrogen) was 2 or 4 ml kg^{-1}, equivalent to 4×10^7 or 8×10^7 leukocytes kg^{-1} respectively.

‡ Fever index (F.I.3) mean ± s.e.

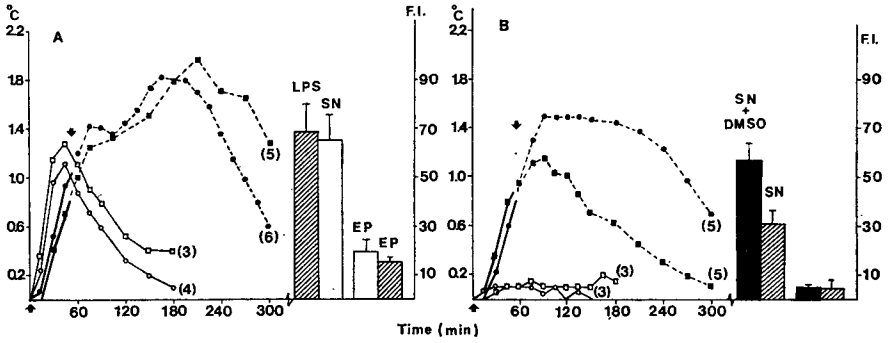

FIG. 1A. Mean increase in rectal temperature (°C-left panel) and mean fever indices (F.I.) ± s.e. (right panel) after intranous injection (↑) of LPS *E. coli* 0·1μg kg^{-1} (■) or sodium nucleate (SN) 40mg kg^{-1} (●). The animals were bled (↓) after 60 min. The plasma obtained was passively transferred to LPS-tolerant animals and assayed for its EP activity: (□) plasma from LPS donor rabbits; (○) plasma from SN donor rabbits. Number of animals in parentheses.

B. Mean increase in rectal temperature (°C-left panel) and mean fever indices (F.I.) ± s.e. (right panel) of febrile rabbits with (●) and with (■) pretreatment with DMSO. The rabbits were pretreated with 25% v/v DMSO, 2ml kg^{-1} intravenously 15 min before intravenous injection of sodium nucleate (SN) 1mg kg^{-1}. The animals were bled (↓) after 60 min, and the plasma obtained assayed for its EP activity: (○) plasma from SN+DMSO donor rabbits; (□) plasma from SN donor rabbits. Number of animals in parentheses.

glassware and solutions free of bacterial pyrogen contamination, for isolating EP, for isolation of peritoneal exudate cells, for preparing LP and for fever testing in the recipient animals have been described in detail by van Miert & Atmakusuma (1971) and van Miert, van Essen & Tromp (1972).

The fever curves were plotted and a fever index (F.I.) calculated (exogenous pyrogens: F.I.5 and endogenous pyrogen: F.I.3); this being the area in square cm under the fever curve during 5 and 3 h of measurement respectively.

The 25% v/v DMSO solution was not contaminated with pyrogens. Pretreatment with DMSO caused a significant increase in fever response to exogenous pyrogens instead of an antipyretic effect (Table 1, Fig. 1B). Unlike exogenous pyrogens, the fever response to LP was not changed after a pretreatment with DMSO (Table 1). LP could be clearly differentiated from exogenous pyrogens by the nature of the febrile response which each elicited when injected intravenously. LP (and EP) evoked a febrile response characterized by a shorter latency time, a more rapid rise to the peak height, a monophasic temperature curve and a quicker drop to the initial temperature level than that produced by exogenous pyrogens. Moreover LP and EP are pyrogenic in exogenous pyrogen-tolerant animals. It is possible that the exogenous pyrogen potentition observed after pretreatment with DMSO is the result of an increase in endogenous pyrogen production as a consequence of better tissue distribution of the exogenous pyrogen. This possibility has been studied. Donor rabbits were given an injection of LPS *E. coli* (0·1 μg kg^{-1}: F.I.5 = 69 ± 11) or sodium nucleate (40 mg kg^{-1}: F.I.5 = 65 ± 10) intravenously and then bled after 60 min. The plasma obtained was passively transferred to LPS-tolerant animals and assayed for its endogenous pyrogen activity. The presence of circulating EP could be demonstrated (Fig. 1A). However, after small doses of exogenous pyrogens this was not possible (Fig. 1B). Pretreatment with DMSO (Table 1, Fig. 1B) effectively potentiated the febrile responses due to small doses of LPS *E. coli* (0·01 μg kg^{-1}: F.I.5 = 56 ± 5·7) or sodium nucleate (1 mg kg^{-1}: F.I.5 = 62 ± 13) but again, endogenous pyrogen (EP) could not be detected in the plasma obtained from these animals. Therefore, it is unlikely that DMSO potentiates exogenous pyrogens by an increase in endogenous

pyrogen production. On the other hand, it has been demonstrated that small amounts of LPS microinjected into the anterior hypothalamus or pre-optic area, produced a long-lasting fever (Myers, Rudy & Yaksh, 1971). Therefore, the exogenous pyrogen potentiation by DMSO is probably related to a better transport of the pyrogen into the central nervous system. This hypothesis is open to further research.

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Body temperature control and arterial gases during halothane anaesthesia in the rat

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For metabolic studies we wished to maintain rats under halothane for up to 4 h with minimal disturbance to their core temperatures, or arterial Po_2, Pco_2, and pH values. Our findings seem relevant to the conduct of any experiment with rats under halothane. It was already known that halothane inhibited heat production in rats (Nikki & Tamisto, 1968).

Experiments were carried out on male, Wistar albino rats, 221–265 g, fully acclimated to 20° ambient temperature. Halothane–air–oxygen mixtures were prepared as described by Sebesteny (1971), but with the halothane reservoir kept at 11–12°. Halothane concentrations were calculated from the vapour pressure–temperature equation (Hill, 1972) and the flow rates of the gases. Anaesthesia was induced by 10 min exposure in a dessiccator to 2·2–2·5% (v/v) halothane. After induction each rat was placed with its head in a mask. Its ambient temperature was measured by mercury thermometers placed alongside it and controlled by the positioning of lamps. Its core temperature was monitored by a thermocouple in the colon. Blood sampling was usually from a tail artery (Agrelo & Dawson, 1968), but sometimes via a carotid cannula introduced 3–6 days earlier (Popovic & Popovic, 1960). Blood removed was immediately replaced by an equal volume of 0·9% saline. Each rat received 300–400 i.u. of heparin parenterally shortly before the first blood sample was taken. Po_2 values could not be maintained in the normal range unless the anaesthetic mixture contained extra oxygen, and the mixture was always enriched by 5–6% (v/v). The halothane concentration required just to maintain stage III anaesthesia, as shown by the suppres-