J. Pharm. Pharmacol., 1965, **17**, 761–765 Received July 2, 1965

Research Papers

The role of the nervous system in local inflammatory responses

H. J. FEARN, S. KARADY* AND G. B. WEST

Inflammation produced in rats by three means has been investigated. The response produced by the external application of xylol seemed to be the result of stimulation of the axon reflex in the area of skin where the substance was applied; it was prevented by chronic denervation of the sensory nerves. The inflammatory response produced by dextran involved the release of 5-hydroxytryptamine; it was prevented by the prior treatment with specific antagonists. The inflammatory response produced by heat treatment did not involve the axon reflex and appeared to be the result of another mechanism, probably involving bradykinin.

WHEN dextran is injected into rats, an acute inflammatory response results. This is called the anaphylactoid reaction and is characterised by pruritus and gross oedema of the extremities. When the hind-paws of rats are immersed in a water-bath at 45° for 30 min, a similar oedema develops below the tibio-tarsal articulation (Rocha e Silva & Antonio, 1960). The external application of xylol also produces oedema with leakage of blood proteins (Aschheim & Zweifach, 1964). We have now examined the role of the nervous system in these three inflammatory responses.

Experimental

METHODS

Groups of at least 5 male Wistar albino rats, 150–200 g, obtained from Bengers, Ltd., Holmes Chapel, were used in each experiment. Either they were injected intravenously with dextran (180 mg/kg) when their hind-paws were immersed in a water-bath at 45° for 30 min after light pentobarbitone anaesthesia (40 mg/kg intraperitoneally), or xylol (2 drops) was applied to each of their hind-paws. All animals received azovan blue dye (10 mg/kg intravenously) 10 min before each of these procedures. The rats were then carefully watched during the next 6 hr and records were made of the degree of oedema formation and of blueing, using an arbitrary scale of 0 to +++.

Surgical procedures. For the local acute denervation experiments, the sciatic and femoral nerves of the animals under ether anaesthesia were cut above the knee and the animals were used either immediately afterwards or about 3 hr later. Chronic denervation experiments were similarly made and the animals were used 3 weeks after the operation when visible signs of degeneration of the nerve fibres such as loss of muscular tone and of claws were prominent.

From the Department of Pharmacology, School of Pharmacy, University of London, Brunswick Square, London, W.C.1.

*Guest worker from the Department of Pathophysiology, Medical University, Szeged, Hungary.

Decerebration was carried out by the dorsal approach under ether anaesthesia; artificial respiration was not necessary and the blood pressure was well maintained at about 80 mm Hg. Pithing of the central nervous system was done by inserting a metal probe through the right eye under ether anaesthesia and then maintaining life with artificial respiration. Spinal transection was done under ether anaesthesia, after exposing the spinal cord at the required level, by cutting the cord with scissors; the blood pressure in these rats was also well maintained, the average value being 70 mm Hg.

Adrenalectomy was done under ether anaesthesia using the dorsal approach; 4 days later, the animals were either tested with the inflammatory agents or they were decerebrated or pithed. In a few experiments, the spinal cord of adrenalectomised animals was transected. Adrenaline (0·5 mg/kg) or noradrenaline (4 mg/kg) was injected subcutaneously 30 min before the inflammatory response was initiated. Other animals received the prior injection of adrenaline, 1 μg, or noradrenaline, 10 μg, intradermally, or of cocaine, 5 mg/kg, either subcutaneously into the paws to anaesthetise the sensory nerve endings or intramuscularly high up the thigh to produce infiltration block, or of cortisone (25 mg/kg subcutaneously).

Results

DEXTRAN RESPONSE

This response was maximal about 10 min after the injection, both oedema and blueing giving maximal scores (+++ results). It was modified by some of the surgical procedures; for example, decerebration, pithing or spinal transection each prevented its development when the dextran was injected immediately after the surgery, yet no inhibition was found when the response was tested 3 hr after surgery. As these surgical procedures did not prevent the dextran response in adrenalectomised rats even when the injection was made as soon as possible after the surgical procedure, it is possible that stimulation of the sympathetic nervous system and of the adrenal cortex occurred during and immediately after the surgery, the released adrenaline, noradrenaline and cortical hormones preventing the reaction. Furthermore, subcutaneous injections of adrenaline, noradrenaline or cortisone prevented the dextran reaction. Neither anaesthesia of the sensory nerve endings by cocaine nor infiltration nerve block modified the response. These results are shown in Table 1.

THERMAL RESPONSE

This inflammatory reaction developed during the heating and was maximal about 1 hr later. Many petechiae and much salivation were observed and oedema was intense. As with the dextran response, decerebration, pithing and spinal transection, each inhibited the thermal response when this was tested immediately after the surgical procedure.

Again, this inhibition may have been the result of stimulation of the sympathetic nervous system and of the adrenal cortex since it was not found when the test was made 3 hr later. Furthermore, no inhibition was found in adrenalectomised animals. Subcutaneous injections of adrenaline, noradrenaline or cortisone prevented the thermal reaction as effectively as they did the dextran response. Cocaine was without effect (see Table 1).

TABLE 1. EFFECT OF VARIOUS PROCEDURES ON THE LOCAL INFLAMMATORY RESPONSES PRODUCED BY DEXTRAN, HEAT OR XYLOL. Groups of 5 rats were used in each experiment. Results and expressed as 0 (= no effect) and + (= prevention of response).

Procedure	Agent producing local inflammatory response		
	Dextran	Heat	Xylol
Acute denervation	0	0	0
Chronic denervation	0	0	+
Cocaine (subcutaneously)	0	0	+
Cocaine (intramuscularly)	0	0	0
Acute decerebration	+	+	Reduced
3 hr after decerebration	0	0	0
Acute pithing	+	+	Reduced
3 hr after pithing	0	0	0
Acute spinal transection	+	+	Reduced
Spinal transection (3 hr later)	0	0	0
Adrenalectomy	0	0	0
Adrenalectomy + decerebration	0	0	0
Adrenalectomy + pithing	0	0	0
Adrenalectomy + spinal transection	0	0	0
Adrenaline or noradrenaline	+	+	+
Cortisone	+	+	0

XYLOL RESPONSE

Within 5 min of application of the xylol, the blueing response was maximal although the oedema reaction did not reach maximal values until about 15 min later. In contrast to the dextran and thermal responses the response resulting from xylol was prevented by chronic denervation and by local anaesthesia of the sensory nerve endings by cocaine (see Table 1). This evidence shows that the peripheral nervous system plays a role in this response but only so far as the intactness of the axon reflex (Jancsó, 1964). The xylol response was unaffected by adrenalectomy (made 4 days previously), but intradermal (and occasionally subcutaneous) adrenaline or noradrenaline inhibited it at the injection sites. Acute decerebration, pithing or spinal cord transection only slightly reduced the response whereas intramuscular cocaine had no action.

Discussion

Inflammation from chemical injury such as the external application of xylol probably depends upon the intactness of the sensory nerve endings, and the initial leak of protein from the blood vessels which is followed some 10 min later by the leak of fluid appears to be the result of stimulation of the axon reflex. The evidence for this is that the xylol reaction is prevented by chronic denervation of the sensory nerves and by cocaine

infiltration of the sensory nerve endings, but not by acute local denervation of the area to which the solvent is applied, by cocaine infiltration of the sensory nerve trunks, or by the procedures of pithing, decerebration or transection of the spinal cord. Intradermal injections of adrenaline or noradrenaline also prevent the xylol response but the mechanism of this antagonism is not clear since noradrenaline, the more potent pressor amine, is nearly 10 times less active than adrenaline. Similar relative activities have recently been reported by Brown & West (1965) who described the antagonism of the intradermal bradykinin and dextran responses in rats.

The topical application of xylol produces, in addition to the inflammatory response, an immediate and pronounced rise in blood pressure. This pressor response is further evidence of the involvement of the nervous system as it is abolished by acute and by chronic denervation of the hindpaws, by pithing, decerebration and spinal section, and by subcutaneous cocaine. Adrenalectomy does not modify this pressor response.

Inflammation from thermal injury or dextran shock, on the other hand, did not depend on intact sensory nerve endings, and the axon reflex probably is not involved. Cocaine infiltration of the sensory nerve ending or chronic denervation are without effect. Nevertheless, in acute experiments, the surgical procedures of pithing, decerebration or transection of the spinal cord much reduced and often completely prevented these two inflammatory responses, although both were well elicited 3 hr later. It is possible that these procedures stimulated the sympathetic nervous system and the adrenal cortex. Both the thermal and dextran responses are prevented by prior treatment with adrenaline, noradrenaline or cortisone.

The primary event in chemical injury is therefore a stimulation of the axon reflex, resulting firstly in a leak of protein from the blood vessels, and later, a leak of fluid after the permeability of the capillaries has been increased by released substances. On the other hand, the primary event in both dextran and thermal shock is the formation and release of vaso-active substances such as bradykinin, histamine and 5-hydroxytryptamine (5-HT), which act directly on the blood vessels leading to a leak of blood protein and fluid into the tissue spaces. Evidence has already been presented that 5-HT is involved in dextran shock (Parratt & West, 1957) and further observations during the present work confirm this result. For example, 2-bromolysergic acid diethylamide, a specific antagonist of 5-HT, in doses of 2 mg/kg intravenously; cyproheptadine, 2 mg/kg intravenously; and reserpine, a liberator of 5-HT, in doses of 2·5 mg/kg intraperitoneally, were all effective in preventing the dextran oedema reaction. On the other hand mepyramine, a specific antagonist of histamine, in doses of 10 mg/kg intravenously, was without effect. Atropine, 100 mg/kg intraperitoneally, and acetylsalicylic acid, 1000 mg/kg orally, also prevented the dextran response, but heparin 10 mg/kg intravenously, had no effect. All of these agents prevented the development of the thermal reaction which probably involves only bradykinin formation and release (Gecse, Karady, Starr & West, 1965).

References

Aschheim, E. & Zweifach, B. W. (1964). *2nd Europ. Conf. Microcirculation*, Pavia, 1962, *Bibl. anat.*, **4**, 315–324.
Brown, R. A. & West, G. B. (1965). *J. Pharm. Pharmacol.*, **17**, 119–120.
Gecse, A., Karady, S., Starr, M. S. & West, G. B. (1965). *J. Physiol. Lond.*, **178**, 8–9P.
Jancsó, N. (1964). *Acta physiol. Hung.*, **24**, *Suppl.*, 1–2.
Parratt, J. R. & West, G. B. (1957). *Ibid.*, **139**, 27–41.
Rocha e Silva, M. & Antonio, A. (1960). *Med. exp.*, **3**, 371–382.