Action of Hypophysectomy and Plasma Transfusion on Anaphylactoid Edema

It is widely accepted that the susceptibility of rats to anaphylactoid edema (AE) induced by certain polysaccharides (such as ovomucoid or dextran) is genetically determined, and that there are strains completely refractory to the damaging action of these substances[1,2]. However, the mechanism of the AE has not yet been clarified. It has been reported that adrenalectomy increases[3] whereas hypophysectomy diminishes or leaves unchanged[4,5] the sensitivity of rats to AE. Recently, we have observed that hypophysectomy inhibits AE induced by iron dextran. Hence we wanted to determine the effect upon AE susceptibility in the absence of the pituitary of plasma transfusion from normal, hypophysectomized or genetically resistant animals.

Fifty Sprague-Dawley rats (sensitive to the AE) with a mean initial body weight of 200 g (range 189–207 g) were treated as shown in the Table. Donors and receptors were hypophysectomized through the parapharyngeal route on the first day of the experiment. Iron dextran 'Fe-Dex' (Imferon®, Fisons Co. of Canada Ltd.) was given i.v. on the 15th day of the experiment at a dose equivalent to 10 mg of inorganic iron in 1 ml of water. This compound (which contains dextran of a very low molecular weight) was used in order to observe the distribution of iron histochemically[6]. Immediately before the Fe-Dex injection, the animals of groups 3, 4, 5 received a transfusion of 4 ml of plasma from Sprague-Dawley untreated rats (group 3), Sprague-Dawley hypophysectomized rats (group 4) and resistant untreated rats of the Wistar-Furth strain (group 5)[7]. The plasma was prepared fresh using the following technique: aorta puncture, collection of heparinized blood and subsequent centrifugation for 10 min at 2000 rpm. Macroscopically, the intensity of AE was graded using a scale of 0–3 in which 0, no edema; 1, just visible; 2, moderate and 3, maximal edema. Two h after Fe-Dex injection, specimens of anaphylactoid shock organs (lips and paws) and dorsal skin from 5 animals/group were fixed in alcohol-formol (4 parts of absolute alcohol and 1 part of 10% neutral formalin) for subsequent embedding in paraffin and staining with the Prussian blue method for iron and with cresyl violet for demonstration of metachromatic material.

As can be seen in the Table, the animals injected with Fe-Dex developed an AE which was visible about 15 min after the injection, already maximal after 30 min and still present 2 h later. Histologically, the anaphylactoid shock organs showed a moderate degree of edema, mast cell discharge, deposition of iron in the extracellular space (either free or linked to discharged mast cell granules) and in the macrophages[6]. The dorsal skin showed neither edema nor deposition of iron; here, the metal was visible only in the lumen of the vessels. The shock organs of hypophysectomized animals did not show any edema on macroscopic observation and histochemically we noted some iron deposition which was mostly located in the vascular walls and macrophages of connective-tissue with little or no mast cell discharge and no iron in connection with mast cell granules. The hypophysectomized rats injected with the plasma of Sprague-Dawley untreated rats (group 3) showed macroscopic and histologic changes similar to those of controls (group 1). The hypophysectomized rats injected with the plasma of hypophysectomized animals (group 4) as well as rats hypophysectomized and injected with the plasma of Wistar-Furth resistant rats (group 5) had lesions comparable to those of the hypophysectomized group (group 2). In control experiments (not reported here) we have seen that the transfer to normal animals of the plasma from untreated resistant or hypophysectomized sensitive rats, does not change their reactivity to Fe-Dex. Preliminary experiments have also shown that the administration of the same amount of plasma as used in our experiments can overcome the inhibition of AE produced by glucocorticoids (Triamcinolone, Aristocort®, Lederle Laboratories Division).

These results indicate that in Sprague-Dawley rats, the susceptibility to AE caused by Fe-Dex is lessened by hypophysectomy and that the transfusion of plasma from normal animals restores the anaphylactoid reactivity. The plasma of resistant rats does not restore the reaction, whereas the plasma of hypophysectomized sensitive animals exerts this action in a limited degree. A possible interpretation of our results is that a substance present in the plasma of normal Sprague-Dawley animals has a permissive action on AE. Such a factor would not be present in resistant animals and its level would be lowered in sensitive animals after hypophysectomy. It is unlikely (although not to be excluded) that it is a hypophyseal hormone since the plasma of untreated resistant animals does not restore the reaction. It is perhaps pertinent that in the rat, dextran reacts with a plasma protein to produce a substance which acts on mast cells to release histamine[8]; furthermore, allegedly, there are reagins in the serum of normal albino rats sensitive to egg-white[9]. It has also

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Group	Treatment[a]	Anaphylactoid edema					
		30 min		1 h		2 h	
		lips	paws	lips	paws	lips	paws
1	None	2.0	2.1	1.8	1.8	1.4	1.3
2	Hypophysectomy	0.2	0	0.2	0	0.1	0
3	Hypophysectomy + plasma of normal animals	1.4	2.0	1.6	1.9	1.3	1.6
4	Hypophysectomy + plasma of hypophysectomized animals	0.5	0.6	0.5	0.7	0.1	0.1
5	Hypophysectomy + plasma of resistant animals	0.2	0.2	0.1	0.1	0.1	0.1

[a] In addition, all animals received iron dextran in a dose equivalent to 10 mg of iron i.v. at 0 h.

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been reported that after the injection of dextran, a compound is formed in the blood which differs from dextran and which, once transferred to normal animals, induces the AE[10]; however, there is some disagreement on this point[11].

It appears that hypophysectomy inhibits the AE and that the reactivity to Fe-Dex is restored by the transfusion of plasma of normal but not of hypophysectomized or resistant animals[12].

Résumé. Chez le rat albino de souche Sprague-Dawley, l'ablation de l'hypophyse empêche le développement de l'œdème anaphylactoïde provoqué par le dextran ferrique. Après hypophysectomie, la transfusion de plasma d'un rat normal de la même souche rend l'animal de nouveau sensible à l'agent anaphylactoïde. Cet effet n'est pas obtenu par la transfusion de plasma d'un rat Sprague-Dawley hypophysectomisé ou d'un rat de souche Wistar-Furth, qui est insensible à l'action anaphylactoïde du dextran ferrique.

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Presynaptic Inhibition in Crustacean Muscle: Axo-axonal Synapse

Most crustacean muscles are innervated by one or more inhibitory axons which regulate muscle tension. When active, the inhibitors weaken or abolish the contractions set up by the motor axons[1]. The inhibition may be either postsynaptic (i.e. the inhibitory transmitter substance acts directly on the muscle fibre membrane), or presynaptic (i.e. the inhibitory action is on the presynaptic terminal of the motor axon). Both effects occur simultaneously in some muscles[2]. In crustacean material the presynaptic effect has been demonstrated in only a few leg muscles of crayfish and crabs, although presynaptic inhibition is known in other material, particularly the mammalian central nervous system[3]. The phenomenon was first described in crustaceans by DUDEL and KUFFLER[2], who found that an inhibitory impulse timed to arrive 1–6 msec before a motor impulse reduced the quantal release of the excitatory transmitter substance while leaving the quantum size unchanged. Subsequent work[4–8] has supported the hypothesis[2] that the inhibitory effect arises from a reaction of the inhibitory transmitter with receptors on the motor axon terminal. The reaction induces an increase in chloride ion permeability of the axon membrane and a consequent decrease in amplitude of the motor impulse in the axon terminal. This reduced 'nerve terminal potential' (n.t.p.) is presumably less effective in releasing the excitatory transmitter.

So far the morphological basis for the effect has remained undisclosed. It could arise from inhibitory synaptic contacts on the motor axon terminal, or from leakage of transmitter from inhibitory neuromuscular synapses to adjacent motor axon terminals. All that can be said from previous histological evidence is that the inhibitory and excitatory nerve terminals occur close together[9]. In this report we present electron microscopical evidence for the existence of inhibitory synaptic contacts on the motor axon terminal.

The electrical manifestations of crustacean presynaptic inhibition are illustrated in Figure 1. The top traces in both records were obtained with an extracellular microelectrode placed close to an excitatory neuromuscular synapse to record the motor n.t.p. and the subsequent flow of current into the postsynaptic membrane (measured as a potential drop in the extracellular solution). The bottom traces show the postsynaptic potentials recorded across the muscle fibre membrane with an intracellular microelectrode. The recording methods are similar to those of DUDEL and KUFFLER[2]. Stimulation of an inhibitory axon (B) produces an inhibitory n.t.p., together with reduction of the excitatory postsynaptic potential, the motor n.t.p., and the synaptic current (compare A and B). The last 2 effects are seen only when the inhibitory n.t.p. precedes the motor n.t.p. by 1–6 msec.

Muscle fibres and attached nerves from preparations manifesting presynaptic inhibition, as shown in Figure 1, were fixed for electron microscopy 1 h in 4% glutaraldehyde in Millonig phosphate buffer, then washed in the buffer alone for 2 h and post-fixed 1 h in 1% buffered osmium tetroxide. The material was dehydrated in an acetone series and embedded in Durcupan (Fluka AG). Sections 50–150 nm in thickness were cut serially from regions thought to contain motor synapses, stained with ethanolic uranyl acetate, and examined in a Phillips EM 200 electron microscope.

Two types of nerve terminal were seen: 1 with predominantly round vesicles of fairly uniform size and 400–500 Å in diameter; and another with vesicles of slightly smaller size (often 200–400 Å) and less regular shape – sometimes round, but quite often elliptical, pear-shaped, or indented (Figure 2).

In the first type of ending, about 90% of the vesicles were circular in cross section, but in the second type, only 40–50% had this property. A statistical comparison of the 2 populations of vesicles was made by measuring the

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