On the mechanism of immunosympathectomy[1]

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Summary. The effects in vivo and in vitro of the antiserum to mouse nerve growth factor (NGF) are independent of the presence of complement. These results are consistent with the view that the antiserum acts by neutralizing endogenous NGF.

Nerve growth factors (NGFs) occur in high concentrations in the salivary glands of adult male mice and in snake venoms[2]. When injected into new-born mammals the antiserum to the mouse factor produces a reduction in the size and number of the neurons in most of the developing sympathetic ganglia[2,3]. This phenomenon is known as immunosympathectomy and 2 theories have been proposed to explain the effect[4,5]. The first postulates that the antibody combines with and deprives the animal of endogenous NGF essential for the survival of the target cells. The second contends that the antibody produces a complement-mediated cytolysis of these cells. There is currently little evidence in support of either model[6]. We now wish to report the results of experiments which examine these possibilities.

Materials and methods. NGFs and antisera were prepared as previously described[7-9]. Pretreatment experiments in vitro were carried out according to our original design[10]. New-born mice were treated with snake NGF, mouse antiserum (Wellcome 8849/46) or cobra venom factor (CoF, Cordis Europa Ltd.) according to the schedules noted. Effects were assessed by weighing the superior cervical ganglia[11]. Plasma levels of complement component C3 were measured by rocket immunoelectrophoresis using a 1% agarose gel containing 20% (v/v) sheep anti-mouse C3[12].

Table 1. Effects of various pretreatments on the response of mouse superior cervical ganglia to NGF

Treatment	Response
CON → CON	0.50 ± 0.13 (13)
M → CON	1.40 ± 0.10 (5)
S → CON	1.80 ± 0.11 (6)
CON → M	1.40 ± 0.19 (5)
M(AS) → M	1.60 ± 0.14 (7)
M(AS),C → M	2.50 ± 0.22 (5)
M(AS),C,M → M	2.30 ± 0.17 (6)
S(AS) → M	2.60 ± 0.19 (7)
S(AS),C → M	1.80 ± 0.17 (6)
S(AS),C,S → M	2.50 ± 0.16 (5)
M → M	2.90 ± 0.22 (8)
S(AS),C,M → M	2.70 ± 0.33 (6)
S → M	3.07 ± 0.19 (7)
M(AS),C,S → M	3.50 ± 0.22 (5)

CON, Control medium; M,S, NGFs (1 BU ml^{-1})[2] from mouse salivary gland and snake venom *(Vipera russelli);* M(AS),S(AS), Antisera (60fold dilution) to the indicated NGFs. This dilution totally inhibits the effects of homologous but not heterologous antigen[9]. C, Complement (10fold dilution of whole guinea-pig serum, Miles). Ganglia were preincubated for 24 h under conditions in which no growth was possible and then transferred to plasma clots and maintained for a further 24 h. Fibre growth was then scored on the accepted arbitrary scale[2] and is an index of the effect of the pretreatment. Values are given as mean scores ± SEM for the number of observations noted. Additives in the pretreatment and final media are indicated according to the key. Thus, M(AS),C,S → M, denotes ganglia pretreated with a medium containing mouse antiserum, complement and snake NGF and then transferred to a clot containing mouse NGF.

Results and discussion. The effect of pretreatments in various media on the subsequent response to NGF of superior cervical ganglia from new-born mice is shown in table 1. Maximum fibre growth occurred when NGF was present in both the pretreatment and final media, intermediate and minimum growth respectively occurred when NGF was absent from one or both media. Preincubation with antisera, with or without complement and NGF, did not significantly reduce subsequent fibre outgrowth. The antibody, therefore, has no demonstrable cytotoxic effect in this system whereas omission of NGF causes marked and obvious effects. The results of various treatments in vivo are shown in table 2. Injection of antiserum into mice (DBA/2J) genetically deficient in complement component C5 and therefore totally devoid of circulating haemolytic activity[13,14], reduced the weight of the test ganglia to the same extent as in normal animals. Similar results were obtained with mice (Porton albino) depleted of component C3 by treatment with CoF[12,15], although plasma levels of this component (measured at day 3 post partum) could not be reduced to less than 25% of the controls. However, levels

Table 2. Effects of various treatments in neonatal mice

Experiment	Wet weight of paired superior cervical ganglia/µg
I. Effect of antiserum in DBA/2J mice	
Control	243 ±26.4 (9)
Antiserum	62.3± 5.1 (8)
II. Effect of cobra venom factor (CoF)	
Control	417 ± 26.5 (4)
CoF alone	396 ± 20.7 (4)
Antiserum	100 ± 18.4 (4)
Antiserum + CoF	111 ± 12.0 (4)
III. Effect of mouse antiserum + snake NGF	
a) 1 ml antiserum + 32 BU NGF	
Control	301 ± 19.9 (4)
Antiserum alone	96 ± 15.7 (4)
NGF alone	340 ± 18.9 (5)
Antiserum + NGF	186 ± 14.9 (5)
b) 0.5 ml antiserum + 2000 BU NGF	
Control	316 ± 18.5 (6)
Antiserum alone	134 ± 7.7 (6)
NGF alone	1137 ± 45.4 (6)
Antiserum + NGF	1156 ±100 (6)

Experiment I: Experimental animals were injected s.c. (50 µl on day 1 post partum and 100 µl on day 2) with mouse antiserum and killed on day 10. Experiment II: Animals were given a single s.c. injection (100 µl, day 1 or 2 post partum) of mouse antiserum. CoF (5 units on days 1 and 2 post partum and thereafter on alternative days) was given i.p. Animals were killed on day 12. Experiment III: Animals were given 5 daily s.c. injections of mouse antiserum and NGF from the venom of *Ancistrodon rhodostoma*[8]. The schedule began on day 1 post partum and animals were killed on day 7. There is no measurable cross-reactivity between the given antigen and antibody[9]: 1 ml of antiserum will neutralize 6000 BU of mouse NGF and < 3 BU of snake NGF.

All values are means ± SEM for the number of animals noted.

in the latter were much lower (about 10%) than in the adult and further reduction may be particularly difficult. Small amounts of snake NGF injected together with the mouse antiserum partially prevented the destructive effects of the antibody and larger amounts produced a characteristic hypertrophy. Since there is no measurable cross-reactivity between this antibody and antigen[9] (and the amounts used preclude an effect based on a direct interaction between the two, see data in footnote to table 2) the snake NGF presumably replaces and supplements endogenous NGF removed by the antiserum. Our results are therefore consistent with and lend some support to the view that the antiserum normally acts by neutralizing circulating NGF. However, this theory does require a correlation between the potencies in vivo and in vitro of different batches and types of antisera. Such a correlation is claimed by some workers[16] but disputed by others[3,11]. While this discrepancy cannot exclude the postulated mechanism, which remains the most likely possibility, it is conceivable that a previously unconsidered effect is involved. Thus, NGF and its antibody may affect different target cells or the antiserum may promote a cell-mediated cytotoxic effect. Whatever the mechanism, it is however now clear that complement fixation is not involved.

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Uptake of tolbutamide by islets of Langerhans and other tissues

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Summary. 3-H-tolbutamide was distributed in a volume exceeding the space occupied by 14-C-sucrose in islets as well as in liver, kidney, muscle, and fat. In contrast to previous reports, the findings suggest that tolbutamide is not restricted to the extracellular space of islets.

Tolbutamide stimulates insulin release from the islet cells by a mechanism still unknown. It has been suggested that the sulfonylurea triggers the secretory response of the islet cell by altering the conformation of the membrane[1]. The insulin releasing activity of a tolbutamide derivative covalently linked to dextran[2] seemed to support this hypothesis. Recently the conclusions from this result have been questioned, however, as instability of the complex might well account for its insulin releasing potency[3]. Another argument for an extracellular action of tolbutamide was the finding that the agent was restricted to the extracellular space of islets[4] that had been marked by 14-C-sucrose. This finding was surprising for 2 reasons: Firstly, the sulfonylurea glibenclamide fairly exceeded the sucrose space[5] of islets. Secondly, tolbutamide is readily metabolized by the liver, indicating that it was taken up by the liver cell. Thus in the present study the uptake of tolbutamide in excess of the sucrose space into islets was re-investigated and compared to liver, kidney, muscle, and fat.

Materials and methods. 50–100 islets obtained from albino mice by collagenase digestion[6] of the pancreas were incubated for 30 min at 37 °C in Krebs-Ringer buffer containing 0.4% albumine, 0.28 mM H-3 tolbutamide (1.6 µCi/ml) (Hoechst AG, Frankfurt) and 1 mM sucrose (0.6 µCi/ml) (Amersham-Buchler, Braunschweig). In parallel experiments slices of liver, kidney, muscle, and fat from the same mouse were incubated. At the end of the incubation period, the tissue slices were freed from adhering buffer on filter paper and dissolved in TS-1 tissue solubilizer (Zinsser, Frankfurt). The islets were collected on pieces of aluminium foil, and the buffer was carefully removed with the aid of a micropipette. The dissolved samples were added to Instagel scintillator (Packard) and counted in an Intertechnique liquid scintillation counter. The channel ratio was used for the quench correction and the calculation of the H-3 and C-14 fractions. The amount of tolbutamide

Uptake of tolbutamide in excess to the sucrose space

Liver	0.22±0.03
Muscle	0.11±0.01
Kidney	0.24±0.04
Fat	0.25±0.02
Islets	0.10±0.03

Values are given as nmole/µl sucrose space. The results represent means ± SEM of 8 experiments. The incubation media contained 0.28 nmoles/µl tolbutamide.