Effect of insulin on immunological phagocytosis by macrophages[1]

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Summary. It was shown that, in physiological concentrations, insulin enhances, in vitro, the immunological phagocytosis of sensitized sheep erythrocytes by cultured mouse peritoneal macrophages. Insulin seems to stimulate macrophage phagocytosis as a cholinomimetic agonist by increasing the intracellular levels of cyclic GMP.

Insulin receptors have been demonstrated on various types of cells involved in immunological reactions, including monocytes[2,3], granulocytes and cultured lymphoblastoid cells[4], peritoneal macrophages[5,6] and stimulated T lymphocytes[7-9]. The evidence that normal macrophages bear specific receptors for insulin[3,5,6], and the observations that tissue stimulation with this hormone result in elevated intracellular levels of cGMP[10,11], led us to investigate the effect of insulin on immunological phagocytosis by macrophages.

Material and methods. Animals. Random-bred SW adult female mice were used.

Sensitized erythrocytes (E.IgG). A washed suspension (2%) of sheep red blood cells (SRBC) was sensitized with equal volume of a 1:1000 dilution of IgG anti-SRBC (SO245, Cordis Laboratories, Miami), in Hanks' solution.

Drugs. The following substances (Sigma Chemical Co.) were assayed: Insulin (bovine) 10^{-3} M-10^{-15} M, carbamylcholine 10^{-6} M, atropine sulfate 10^{-5} M, theophylline 10^{-3} M, deoxyglicose 50 µg/ml, trypsin 1 mg/ml, phospholipase A and C 100 µg/ml.

Phagocytosis assays. Peritoneal macrophages were harvested with RPMI 1640 medium (DIFCO), supplemented with 10% FCS and 10 U/ml heparin. 0.1 ml of the suspension containing $2 \cdot 10^6$ cells/ml was added to 2 coverslips in 4-cm Petri dishes. After incubation at 37 °C for 60 min, the non-adherent cells were washed.

The coverslips containing the macrophage monolayer were covered with 2 ml of the RPMI medium and cultured for 24 h at 37 °C in an atmosphere of 5% CO_2. For the phagocytosis assays, the coverslips were washed and then covered with 2 ml of the E.IgG suspension containing the drugs. After incubation for 60 min at 37 °C, the coverslips (test and controls) were exposed during 20 sec to Hanks' solution, diluted 1:5 with water to lyse the non-ingested erythrocytes. The macrophages were then fixed with glutaraldehyde, treated with benzidine-H202 mixture and stained by Wright.

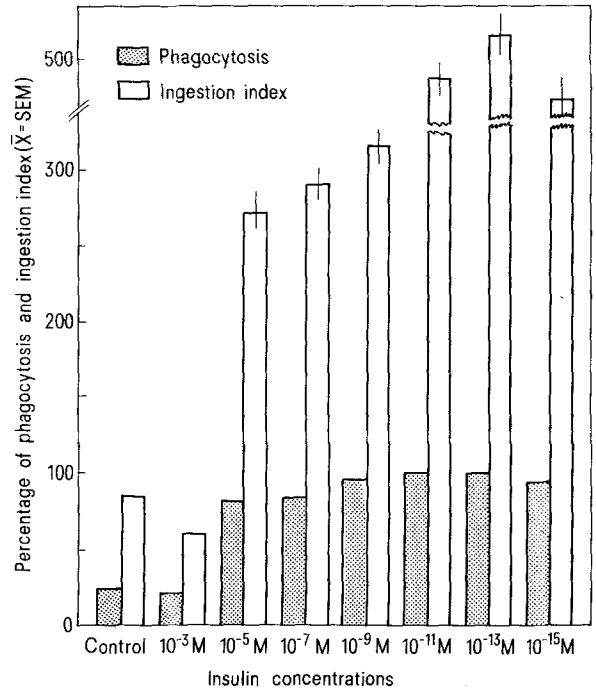
Results. The results obtained showed that in physiological concentration, insulin has enhanced, in vitro, the immunological phagocytosis of SRBC sensitized with rabbit IgG by normal cultured mouse peritoneal macrophages. This enhancement can clearly be seen when the results are analyzed, taking into account the ingestion index and not only the percentage of macrophages engaged in phagocytosis (figure). This effect could be noted within the 1st 60 min of incubation of macrophage with insulin and E.IgG, but it could not be observed with non-sensitized SRBC. The maximum of phagocytosis activation (about 5 × above controls) was observed with cultured macrophages using insulin in concentrations between 10^{-9} M and 10^{-13} M. With larger doses of insulin (10^{-5} M-10^{-7} M), the enhancement of the ingestion index was less pronounced. With stronger concentration (10^{-3} M), there was no significant changes in the ingestion index.

Insulin seems to stimulate macrophage phagocytosis as a cholinomimetic agonist by increasing the intracellular levels of cGMP. Its effect could be completely blocked in the absence of calcium, partially blocked in the presence of atropine sulfate (10^{-6} M) and potentiated by carbamylcholine (10^{-7} M).

Substances capable of blocking the stimulatory effect of insulin on macrophage immunological phagocytosis. Cultured normal peritoneal macrophages from SW adult mice. Phagocytosis of sheep erythrocytes sensitized with rabbit IgG

Substances	Phagocytosis (%)	Ingestion index
Insulin 10^{-11} M	80	320
Insulin 10^{-11} M + trypsin 1 mg/ml	5	6
Insulin 10^{-11} M + phospholipase A 100 µg/ml	40	124
Insulin 10^{-11} M + phospholipase C 100 µg/ml	10	20
Insulin 10^{-11} M + deoxyglicose 50 µg/ml	5	10
Insulin 10^{-11} M + atropine 10^{-5} M	45	220
Insulin 10^{-11} M + theophylline 10^{-3} M	19	48
Insulin 10^{-11} M + serum anti-Mø 1:20*	0	0
Insulin 10^{-11} M + diabetic serum 1:20	12	29
Control	48	223

* Rabbit anti-macrophage serum.



Influence of insulin concentrations on macrophage immunological phagocytosis of sheep erythrocytes sensitized to rabbit IgG. Ingestion index = percentage of macrophages with ingested erythrocytes × average number of erythrocytes ingested per macrophage.

This effect of insulin on macrophages could also be blocked by a serum from a diabetic patient with high insulin resistance and capable of reducing about 80% the uptake of ^{125}I-insulin by macrophages. Several other substances could also inhibit the stimulatory effect of insulin on macrophages: rabbit anti-macrophage serum (1:20 dilution), trypsin (1 mg/ml), phospholipase A and C (100 µg/ml), deoxyglicose (50 µg/ml), theophylline (10^{-3} M) (table).

Discussion. Previous reports[13,14] from our laboratory demonstrated that immunological phagocytosis by mouse peritoneal macrophages can be influenced by drugs which modulate the intracellular levels of cyclic nucleotides (cAMP and cGMP). It was shown that agents known to raise the intracellular levels of cAMP are inhibitors of phagocytosis, while phagocytosis was enhanced by cholinergic drugs which raise the intracellular levels of cGMP. In this paper we demonstrated that in physiological concentrations, insulin enhanced, in vitro, the immunological phagocytosis of SRBC sensitized with rabbit IgG by normal mouse peritoneal macrophages. With higher concentrations, there were no significant changes in the ingestion index. According to our results, insulin seems to stimulate macrophage phagocytosis as a cholinomimetic agonist by increasing the intracellular levels of cGMP. Insulin has been reported to have a physiological effect on macrophage functions, but the results obtained are somewhat contradictory. Rhodes[15], for instance, showed that the macrophage-Fc expression is inhibited by insulin (10 µg/ml or 50 µg/ml), dibutyryl AMP and methylxanthine, but is as augmented by the dibutyryl cGMP. According to Muschel et al.[5], insulin at concentration of 60 ng/ml produced a marked depression in Fc-mediated phagocytosis by mouse peritoneal macrophages, and that its effect was reversed by cAMP, isoproterenol and cholera toxin.

The conclusions that insulin inhibits macrophage-Fc expression[16] and that cAMP enhances and insulin inhibits Fc-mediated ingestion by peritoneal macrophages, are in conflict with our results. We have no explanation for these discrepancies, but at least 2 main technical variants could well be invoked to elucidate them: a) different concentrations of insulin used for attaining maximal effects; b) different methods for evaluating the results obtained. The evaluation of experimental results by taking into account only the percentage of cells engaged in phagocytosis is fallacious. It is also necessary to determine the ingestion index, as can easily be seen from our data.

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Effects of castration, estradiol and testosterone on tubulin levels of the medial basal hypothalamus and the adenohypophysis of the rat[1]

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Summary. Tubulin levels of the medial basal hypothalamus (MBH) were greater in male than in female rats. Orchidectomy brought about a decrease of MBH tubulin concentration, whereas testosterone injection augmented it in the MBH and adenohypophysis. Estradiol administration augmented MBH tubulin and protein concentration.

The biochemical and pharmacological properties of the protein constituting microtubules (tubulin) have been well characterized. Its binding to the antimitotic drug colchicine exhibits a specific and stoichiometric affinity which has provided an assay for estimating tubulin concentration in a number of tissues, including the brain[3]. In this tissue tubulin comprises 15–40% of the total soluble protein and its high concentration has been one of the most significant factors to indicate that microtubules may play an important functional role in nervous tissue. Mainly based upon indirect observations by using agents (e.g., colchicine or vinblastine) which disrupts microtubules, these organelles have been implicated in both axoplasmic transport and neurosecretion[4]. Very few observations have been published concerning changes of colchicine-binding activity of the brain as a function of the neuroendocrine status of the animal. Hypothalamic tubulin was found to be affected by catecholamine transmitter through alpha- and beta-adrenergic receptors, the latter involving the pineal gland[5], and presumably melatonin secretion[6]. Experimental manipulations known to alter the neuroendocrine apparatus, such as continuous exposure to light or superior cervical sympathectomy, also resulted in modification of colchicine-binding activity of the hypothalamus[5]. The present experiments were undertaken to examine the effects of estradiol and testosterone on tubulin levels of the medial basal hypothalamus (MBH) and the adenohypophysis (AH) of castrated rats.

Material and methods. Adult Wistar rats (180–220 g) were kept under controlled lighting from 07.00 to 21.00 h daily