Alopecia in laboratory animals induced by a polyampholyte, polyethylene alanine

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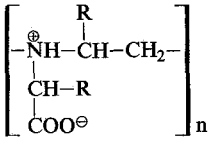
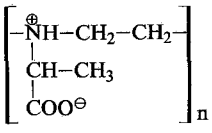
Summary. Polyethylene alanine, a polyaminoacid, was found to induce a characteristic alopecia when administered topically or systemically to suckling mice, or newborn guinea-pigs or rabbits.

As reviewed by Torrence and De Clercq[2], polyanionic substances such as the polycarboxylic acids, polyacrylic acid (PAA) and pyran (maleic anhydride divinylether) copolymer, exert a variety of biologic effects in the organism. They stimulate interferon production, humoral and cell-mediated immunity, and macrophage activity. To a large extent these effects contribute to the inhibition of virus infections and malignant tumor growth that has been observed with these polyanions in various animal model systems[2].

Part of our recent research efforts has been directed towards the synthesis[3,4] of polyampholytes of the general type



where R is either H or CH_3, and to explore these polyaminoacids for their pharmacological properties. One of these polymers, polyethylene alanine (PEA)



was found to provoke a marked hair loss, when administered i.p. to 10-day old NMRI mice. This unexpected finding is further documented in the present study.

All mouse experiments were carried out with randomly bred NMRI mice and all data reported refer to groups of 10 mice. As the mice were only 10-days-old, thus still suckling, when treated, they were housed 10 per cage with their mother.

When PEA was administered i.p. at 1 mg/mouse (~160 mg/kg) to 10-day-old mice, hair shedding was first noticed on the 5th day after injection. Hair loss quickly proceeded (figure 1) and was complete 10–12 days after PEA treatment (figure 2). Hair growth resumed about 14–16 days after PEA injection and 20 days after the injection the fur was almost completely restored (figure 3).

The age of the mice appeared critical for the alopecia to occur. No hair loss was noted if PEA was administered i.p. at doses up to 5 mg/mouse to 20-day-old or adult mice. In 15-day-old mice hair loss was observed in some, but not all, mice which were given PEA (1 mg/mouse) i.v. The hair loss pattern in 10-day-old mice was similar for PEA administered i.p. or s.c.

Even when administered topically, once daily for 5 consecutive days starting at the age of 10 days, PEA [at 5% w/v in a vehicle containing 50% dimethylsulfoxide (DMSO) and 50% phosphate buffered saline (PBS)] induced marked hair loss. This was restricted to the skin area where PEA had been applied. It was not as severe as the alopecia noted with the systemic PEA treatment.

The depilatory effect of PEA was not limited to the mouse. Guinea-pigs and rabbits which had received a single injection i.p. of 100 mg/kg of PEA 1 or 2 days after birth showed slight to massive loss of hair, but this hair shedding only occurred at some localized skin areas (i.e. shoulder, abdomen) and did not spread over the rest of the body surface, as seen in mice.

Extensive dialysis of PEA (for 4 days against 100× vol. PBS) did not affect its depilatory activity, suggesting that this activity resided with the macromolecular structure of the compound.

Figs 1–3. NMRI mice after being treated i.p. when 10 days old with PEA (1 mg/mouse; ~160 mg/kg). Pictures taken on the 5th day (figure 1), 10th day (figure 2) and 20th day (figure 3) after PEA treatment.

The interference of PEA with hair growth cannot be regarded as a merely toxic manifestation of the drug, since, at the dosage employed (~160 mg/kg in 10-day-old mice), PEA did not affect the growth rate of the animals. Neither did it affect white blood cell (WBC) counts (as determined 2, 4 and 8 days after injection of PEA i.p.). Yet, leucopenia is a commonly encountered complication following the administration of defleecing agents such as cyclophosphamide[5].

Skin biopsy specimens were taken at various intervals (5, 10 and 15 days) after 1 mg PEA was injected i.p. into 10-day-old mice. Histopathological examination of the skin specimens (following staining with erythrosin-hematoxylin) revealed an infiltration of the dermis by mononuclear cells (lymphocytes and mast cells). This mononuclear cell infiltration of the dermis was most conspicuous in the skin biopsy taken 5 days after PEA injection, but was still evident in the biopsies taken 10 and 15 days afterwards. The presence of mast cells in the cellular infiltrate is indicative of an inflammatory response.

In the skin fragment collected on the 10th day there was a marked reduction in the number of hair follicles which all occurred in the same (catagen) phase. Some hair shafts were dilated or deformed. The number of hair follicles returned to normal by the 15th day after PEA treatment. In control skin biopsies all hair follicles uniformly occurred in the anagen phase. The timing of hair loss with PEA treatment and its histological appearance may seem compatible with anagen effluvium[6].

There are various medications that are reputed to cause hair loss. These drugs include antimitotic agents such as cyclophosphamide, methotrexate, vincristine, colchicine, anticoagulants such as heparin and coumarins, antithyroid drugs, such as thiouracil and carbimazole, vitamin A, boric acid, thallium and several others[6,7]. Whereas antimitotic agents lead to a characteristic anagen effluvium, thallium and anticoagulants induce telogen effluvium. Some of these depilatory agents, in particular those that cause anagen alopecia, such as cyclophosphamide, have been advocated for defleecing of sheep[5,8,9]. Another agent that effects hair or wool loss is mimosine[10], a naturally occurring amino acid present in the leguminous shrub, *Leucaena glauca;* sheep fed on a sole diet of *L. glauca* will shed their fleece.

Whether PEA has any potential applications as a depilatory or defleecing agent, how it induces alopecia and how its depilatory activity compares with that of structurally related analogs, remain obvious matters for further study.

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Effects of dobutamine on cyclic AMP accumulation induced by the stimulation of dopamine receptors in rabbit retina in vitro

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Summary. Intact rabbit retinae were used for testing in vitro the potential activation of dopamine receptors by a new cardioactive sympathetic amine dobutamine. It was found that despite the structure relationship of dobutamine with other dopamine-analogs, the pharmacological action of this compound is not comparable to that of apomorphine, N-methyl-dopamine and/or ADTN.

Dobutamine is a new synthetic cardioactive sympathetic amine which has been recently introduced in therapeutics (Sonnenblick et al.[5]). As inferred from a variety of pharmacological data obtained mostly in vivo[6,7], it appeared that the cardiovascular effects of this catecholamine resemble those of isoproterenol more than those of dopamine. It stimulated adrenergic β_1-receptors, whereas β_2- and α-receptors were only activated to a small extent[5,6,8]. A possible stimulation of peripheral and/or central dopamine receptors by dobutamine, whose chemical structure is close to that of dopamine analogs, has not been investigated yet, either at cellular or at molecular level. It was therefore of interest to test dobutamine in vitro, in a preparation such as mammalian retina. This tissue contains a population of dopamine receptors coupled to the enzyme adenylyl cyclase and it is a useful and predictive model for testing certain types of potential dopamine receptor agonists[9,10]. It has also been used recently to characterize dopamine receptors by pharmacological displacement of ^{3}H-spiroperidol binding[11].

Materials and methods. The methods employed for the dissection and isolation of the rabbit retina have been described elsewhere[12]. Following a pre-incubation period of 40 min at 35 °C in a Krebs-Ringer medium (pH 7.4), the retinae were cut in half (vertical axis) and submitted to a final incubation of 10 min at 35 °C in the same medium, except that 10 mM theophylline was present. For each experiment, 5 half-retinae, chosen in a random way, were kept as controls. Others (at least 5 for each drug) were submitted to drug-treatment and a comparison was then made of the potency of dobutamine at 10^{-6}–10^{-4} M concentration with that of dopamine and/or dopamine-mimetic agents. In experiments performed with an adrenergic- and/or dopamine-receptor antagonist, it was added to the medium in appropriate dilution (up to 10^{-4} M), before the addition of the agonist for the final 10 min incubation period. Cyclic AMP and proteins were then measured as described[12]. All drugs used were from commercial sources, except N-methyl-dopamine(epinine), which was kindly provided by Dr. P. Laduron (Beerse).