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).

Results and discussion. Figure 1 shows that at 10^{-4} M concentration dobutamine induced a slight but significant accumulation of cyclic AMP. The percent increase over control retinae was, however, much less pronounced with dobutamine (150%) than with dopamine (400%), adrenaline (300%) or noradrenaline (400%), when each of those catecholamines was tested at the same concentration under similar experimental conditions[9]. This stimulatory effect of 10^{-4} M dobutamine upon cyclic AMP formation was suppressed by the concomitant addition to the incubation medium of 10^{-5} M fluphenazine, a selective dopamine receptor antagonist, thus showing a pharmacological specificity of the interaction of dobutamine at high concentrations with dopamine receptors (figure 1). The specificity of dobutamine action was stressed by the fact that phentolamine as well as propranolol used at 10^{-4} M concentrations were unable to inhibit the cyclic AMP increases induced by dobutamine (not shown).

Figure 2 shows, however, that the stimulatory effects of dobutamine, although specific for the activation of dopamine receptors, were not obtained at 10^{-6} M concentration. This concentration corresponds to the ED_{50} of dopamine[13]. As far as a comparison with the effects of other catecholamines is concerned, it has been shown previously that adrenaline, and noradrenaline, were also unable to stimulate the production of cyclic AMP at 10^{-6} M concentration[9], in contrast to significant effects observed at 10^{-4} M[9]. A different feature was found with a dopamine-analog (epinine), a dopamine-mimetic drug (apomorphine) as well as with a new type of dopamine-agonist such as 6,7-dihydroxy-tetrahydronaphtalene (ADTN), which have comparable effects to those of dopamine (figure 2, and Schorderet et al.[10]). It is known from these and previous published data that the potency and the efficacy of these drugs were similar to those of dopamine. A second class of drugs, which stimulate only at 10^{-4} M, appears then to be adrenaline, noradrenaline and to some extent dobutamine (figure 1). Isoproterenol was shown to be totally devoid of dopamine-mimetic activity even at the highest concentration[9].

Natural (endogenous) catecholamines such as dopamine, adrenaline and noradrenaline as well as synthetic compounds such as isoproterenol are responsible for causing some undesirable side-effects when they are used as cardioactive agents[5]. Among newly synthetized compounds, the desoxy analogs of isoproterenol were found to be devoid of chronotropic activity and cardiac automaticity. Thus, catecholamines lacking the side chain hydroxyl group (dopamine analogs) appeared to have less arrhythmogenic activity than the parent compounds. Above all substances tested, dobutamine has emerged as a new potent agent for the treatment of cardiac failure[14]. It shares with dopamine the absence of the β-hydroxyl group, whereas its N-alkyl substitute resembles that of isoproterenol. The pharmacological properties of the new drug should therefore depend upon its dopamine- and/or isoproterenol-moiety. In fact, most studies performed in men and/or in animals in vivo seem to imply that dobutamine is a selective stimulant of β_1-adrenergic receptors[5,15]. The new drug appears, then, to have a potent inotropic action, without chronotropic and peripheral vasodilating effects. On the other hand, dobutamine does not seem to provoke renal vasodilatation[16], which is possibly due to the interaction of a dopamine-mimetic drug with presynaptic dopamine receptors[17]. A few data are available as to the effects of dobutamine at cellular and/or molecular level[8,18,19]. Since a majority of dopamine postsynaptic receptors are coupled to the enzyme adenylylcyclase (D_1-type receptors[20]), numerous pharmacological studies of the action of dopamine and of dopamine-related drugs of the action of dopamine and of dopamine-related drugs have been performed by measuring the cyclic AMP accumulation induced by these agents in various intact or broken-cell preparations of striatum and/or retina[21]. In addition, homogenates prepared from brain and renal arteries for adenylyl cyclase activity studies exhibit considerable similarity in their response to agonists and antagonists[22]. Finally, bovine retina homogenates were also successfully used for pharmacological studies of dopamine receptors based on the displacement of ^{3}H-spiroperidol binding by various dopamine-agonists and/or antagonists[11]. In these experiments, dobutamine was found to be a very weak agent (not shown).

Thus, the present report indicates that dobutamine cannot be considered as a dopamine-mimetic drug like, for example, another type of dopamine-analog such as N-methyldopamine (epinine). It was not able to stimulate, at 10^{-6} M, the formation of cyclic AMP in rabbit retina in vitro. Even some ergot alkaloids, whose chemical structure is far from identical with that of dopamine and which are nevertheless supposed to act as dopamine agonists in vivo, were able at 10^{-6} M concentration to increase the cyclic AMP levels of

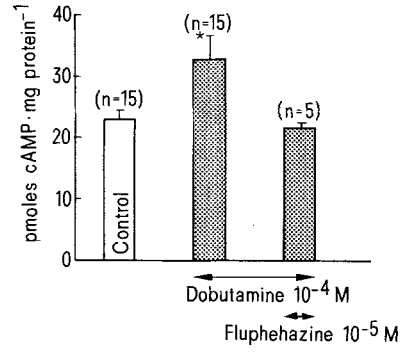

Fig. 1. Effects of dobutamine (10^{-4} M) upon cyclic AMP formation in intact rabbit retinae, in absence or in presence of fluphenazine (10^{-5} M). The final 10 min incubation with control retinae as well as with drug-treated retinae was performed in presence of theophylline (10^{-2} M). Values are means ± SEM for the number of samples given in parentheses. * Different from control, $p < 0.05$, Student's t-test for unpaired data.

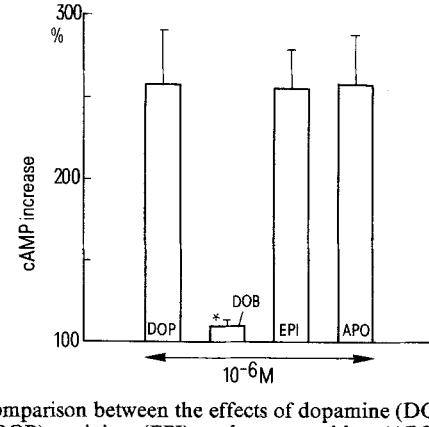

Fig. 2. Comparison between the effects of dopamine (DOP), dobutamine (DOB), epinine (EPI) and apomorphine (APO), used at 10^{-6} M concentration, upon cyclic AMP formation in intact rabbit retinae. The experiments were performed in presence of theophylline (10^{-2} M). Values are means of percent increases (± SEM) over control values (n = 5, each drug). Absolute levels of cyclic AMP were 36.9±4.7 (DOP), 16.6±0.7 (DOB), 38.6±3.6 (EPI) and 39.0±4.6 (APO), whereas cyclic AMP levels in control retinae were 14.3±1.9, as referred to dopamine, and 15.1±1.5, as referred to the 3 other drugs, tested simultaneously. * Not different from control.

rabbit retina in vitro[23]. It is worthy of note, however, that dobutamine, like adrenaline, noradrenaline or an isomer of ADTN (i.e. 5,6-dihydroxy-tetrahydronaphtalen)[9,10] was able to increase cyclic AMP, when it was applied at 10^{-4} M. This concentration of dobutamine is hardly obtained in plasma in vivo, when applied in the range of therapeutic doses advised for man[15]. Nevertheless, in case of accidental overdose, one has to keep in mind that besides its specific β_1-stimulating properties, dobutamine may possibly display some dopamine-mimetic activity which can modify the symptoms of drug intoxication.

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Effects of colchicine, cytochalasin-B and papaverine on wound healing in *Xenopus* early embryos

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Summary. The effects of colchicine, cytochalasin-B and papaverine on wound healing in *Xenopus* early embryos have been studied. Colchicine does not prevent wound healing, whereas cytochalasin-B does. Papaverine, under conditions which prevent the completion of neurulation, does not prevent wound healing. A model is given which might explain these observations.

Amphibian embryos have been used extensively for studies in experimental embryology, partly because they show a remarkable ability to heal following manipulation. However, until recently there has been little systematic study of wound healing in amphibian early embryos, although a number of observations have been made en passant. For example, gaping of a wound made in amphibian neurulae has shown that the ectoderm is under lateral tension[2]. Also, it has been noted that healing of the wound following micro-injection of amphibian gastrulae was inhibited by cytochalasin-B[3], although it is difficult to determine the effective concentration of cytochalasin-B in this case. Recently, a scanning electron microscopical study of wound healing in *Xenopus* neurulae has shown that following initial gaping of the wound, the cells surrounding the wound become tapered towards the cut edge[4]. This observation is compatible with the idea that wound closure is effected, at least in part, by coordinated changes in cell shape. The aim of the present work has been to attempt to determine the mechanisms by which such changes in cell shape are effected, by studying the effect of inhibitors on wound healing. Colchicine is thought to disrupt microtubules[5], which have been observed in a number of developing systems where morphogenesis is occurring[6]. Cytochalasin-B is thought to reversibly disrupt microfilaments[7], which have been observed in amphibian gastrulae[8] and neurulae[9], and has been shown to inhibit gastrulation in *Xenopus*[3]. Papaverine has been shown to prevent neurulation in amphibian embryos[10], which is accomplished by changes in cell shape similar to those described in wound healing[6], and it has been suggested that papaverine acts by inhibiting calcium fluxes.

Methods. Xenopus embryos were obtained by injecting pairs of adults with chorionic gonadotrophin (Chorulon, Intervet Ltd). The jelly coats were removed by placing the embryos in 2% cysteine hydrochloride in 10% Steinberg saline, brought to pH 7.8 with 2 M NaOH. The embryos were washed and subsequently cultured in 10% Steinberg saline, pH 7.3. When the embryos had reached the late blastula stage (stage 9[11]), the vitelline membranes were removed using Watchmakers' forceps, and the embryos were cultured overnight and subsequently handled over 1% agar. When the embryos had reached the neurula stage (stage 17-18[11]), they were wounded by making a longitudinal incision approximately 0.5 mm long in the lateral ectoderm using an electrolytically-sharpened tungsten needle. The process of wound healing was followed over the next 60 min.

For colchicine treatment, embryos were exposed either to 10^{-3}-10^{-5} M colchicine for 2 h before wounding and during recovery, or to 1.25×10^{-3} M colchicine overnight from the late gastrula stage. In this latter case, a rent was made in the roof of the blastocoel of the gastrula to facilitate penetration of the drug[12]. For cytochalasin-B treatment, a different protocol was used since prolonged exposure to cytochalasin-B causes disaggregation of amphibian embryos. Neurulae were exposed to 0.3-5.0 µg/ml cytochalasin-B in dimethylsulphoxide (DMSO) for 5 min before wounding and during recovery. Controls included embryos wounded in DMSO alone, and intact embryos in cytochalasin-B. For