reactions. The mean weights of the 4 groups did not differ by more than 24 g (overall mean was 181.8 g). However, the initial mean startle reactions of the albino animals were much smaller than those of the pigmented group (Table II). This difference occurred despite the fact that 43 albino rats were screened to obtain the 10 experimental animals, whereas the 10 pigmented animals were selected from just 14 rats tested. Since the stimulus and the test procedure were the same in both cases, this result could imply some inherent difference between the strains of rat in their acoustic startle reaction.

In this experiment, there were no significant reductions in response in either control group (Table II). By the use of paired t-tests, it was shown that there was a significant ($p < 0.05$) reduction in the mean startle reaction of the pigmented drug group at day 36 and a highly significant ($p < 0.01$) reduction at day 101. However, at these times, there were very highly significant ($p < 0.001$) reductions in the mean startle reaction of the albino drug group compared with the initial control value; these significant reductions in startle reaction were taken to have resulted from drug-induced hearing impairment.

These latter results certainly question the hypothesis that, during chronic intoxication with kanamycin, albino animals are less likely than pigmented animals to suffer cochlear lesions resulting in hearing impairment. However, this interesting possibility is being further explored in our laboratories using a more refined method for testing hearing – namely, operant conditioning of tone discrimination. The resolution of this problem could lead to a fuller understanding of the mechanism of toxic action of such drugs on the inner ear.

Summary. Following the finding that melanin pigment played a role in the accumulation of ototoxic drugs in the inner ear, an investigation was made of the possible influence of the pigmentation of animals on their susceptibility to the ototoxic effects of drugs. Hearing acuity was assessed by measurement of acoustic startle reaction. Preliminary experiments suggested that pigmented animals might be more likely to suffer hearing impairment following ototoxic drug administration. However, in a controlled study using rats treated with kanamycin, it was not possible to confirm this and albino animals appeared no less vulnerable than pigmented animals to kanamycin-induced deafness.

E. S. HARPUR[8] and P. F. D'ARCY

Department of Pharmacy
The Queen's University of Belfast,
Medical Biology Centre, 97, Lisburn Road,
Belfast BT9 7BL (Northern Ireland), 11 July 1975.

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The Effects of Dopamine, Piribedil (ET-495) and its Metabolite S-584 on Retinal Adenylate Cyclase

It has been suggested that in rats adenylate cyclase might be the possible dopamine-receptor within the caudate nucleus of the central nervous system[1]. As a result of this, striatal homogenates of various other species, including man, have been used by numerous workers to investigate the mechanism of action of neuroleptic agents, which are supposed to exert their effects by blockade of dopamine-receptors[2–4]. In a broad sense, a correlation between potency of these agents in vitro, as inhibitors of dopamine-sensitive adenylate cyclase, and their effects in vivo as neuroleptics, seems to exist, although some discrepancies have been encountered[2,4]. We have recently found that intact or homogenized retinae of the rabbit may provide another useful system for such investigations in vitro[5,6], since the neuronal catecholamine in the retina of this species seems to be mostly dopamine[7]. The increased selectivity (and sensitivity) of our model, compared with other striatal or retinal preparations[8,9], also makes it suitable for elucidating the mechanism of action of other drugs, such as experimental or clinical antiparkinsonian agents. Moreover, this can be done at the cellular or molecular level. For example, we have recently shown that apomorphine, a typical "dopamine-like" drug, is a very potent dopamine-receptor agonist, in intact cells as well as in homogenates of rabbit retina[5,6]. In contrast, we have been able to demonstrate that the mechanism of action of amantadine, a clinical antiparkinsonian drug, is not related to direct stimulation of dopamine-receptors[5], although other authors have suggested this possibility[10].

Among the antiparkinsonian drugs acting directly on dopamine-receptors, L-dopa is the most specific. Several attempts have been made to synthesize other dopaminomimetics, such as apomorphine, or piribedil (ET-495) [1-(3,4-methylene dioxybenzyl)-4-(2-pyrimidinyl)piperazine], a non-catechol analogue of dopamine. The agonistic activity of the latter seems to be questionable[11], although it has been proposed that its metabolite formed in vivo, namely S-584 [1-(3,4-dihydroxybenzyl)-4-(2-pyrimidinyl)piperazine] can directly activate striatal dopamine-receptors[12]. To test this hypothesis, we have investigated the effects of both piribedil as well as S-584 on either intact cells or homogenates of rabbit retina, under conditions where the activity of the dopamine is maximal.

Methods. The procedure for the dissection of the retina has already been described[5]. When intact retinae were used, the following modifications of the previous experimental procedure[5] were introduced. Oxygenation with 95% O_2–5% CO_2 during the final 10 min incubation (in the presence of drugs) was not applied, in order to maintain the very easily breakable retina in good shape. This

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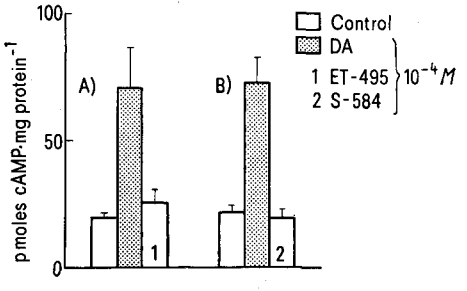
precaution is beneficial for the reproductibility of control as well as stimulated tissue results. However, the physiological medium (Krebs-Ringer bicarbonate) was well gassed before being used for the final incubation, which was carried out in glass-homogenizers containing a total volume of 0.5 ml (instead 1.0 ml) in the presence of 5 mM theophylline. After the 40 min of pre-incubation, the retinae were cut through the vertical axis in 2 equal pieces and distributed in a random way. Each homogenizer was provided with one half of a retina. Instead of adding TCA to the medium, the homogenizers were removed from the bath and quickly plunged in boiling water for 10 min. The tissue was then homogenized, at 4 °C, and centrifugation was performed at the same temperature. The pellets were used for protein determination[5]; 20 µl duplicate samples of the crude supernatant were taken out for the saturation assay[13]. The same volume of buffer (Krebs-Ringer-theophylline) was used for running the standard-curve made in the presence of 0, 1, 2, 4, 8 and 16 pmoles of cyclic AMP. When the experiments were performed on retinal homogenates, the methodology was as previously described[6]. When extraction and purification of cyclic AMP by means of ion exchange chromatography on Dowex 50 W-X 8 is omitted, the sensitivity of the assay in our experimental conditions is decreased. Therefore, where homogenates are concerned, this step in experimental procedure seems to be unavoidable.

Results and discussion. As shown in the Figure piribedil as well as piribedil metabolite S-584 did not raise cyclic AMP levels in isolated retina of the rabbit. In contrast, dopamine at the same concentration (10^{-4} M) was found to be very potent, in accordance with our previous results[5]. It should be mentioned that following the introduction of some methodological improvements (see Methods), the effects of dopamine were even more pronounced than in previous experiments. For example, the Figure shows an increment in cyclic AMP concentrations of more than 50 pmoles over control values, whereas in the previous series of experiments[5] the increment was of 26.2 pmoles. Furthermore, we have recently observed a half-maximal stimulation in the presence of 10^{-6} M dopamine (not shown). On the other hand, isoproterenol at 10^{-4} M concentration was found to be totally ineffective[14]. Thus, unlike rat caudate nucleus which contains both β-adrenergic-receptors (stimulated by isoproterenol and blocked by propranolol) and dopaminergic receptors (blocked by fluphenazine)[15], rabbit retina seems to contain dopamine-receptors exclusively.



Comparative effects of dopamine (DA), piribedil (ET-495) and S-584 on cyclic AMP concentration in isolated retina of the rabbit. Half-retinae have been incubated for 10 min at 35 °C in the presence of 5×10^{-3} mM theophylline. A) and B) represent separate experiments. The data give the mean values + SEM of a minimum of 4 half-retinae.

Comparative effects of dopamine (DA), piribedil (ET-495) and S-584, each at 10^{-4} M concentration, on adenylate cyclase activity in homogenates of rabbit retina[a]

	Basal	DA	ET-495
A)	104.4 ± 1.6	248.0 ± 20.3	76.9 ± 12.4

	Basal	DA	S-584
B)	93.6 ± 8.7	216.3 ± 33.5	92.0 ± 7.1

[a] pmoles cyclic AMP·mg protein^{-1}·2.5 min^{-1} under standard assay conditions. A) and B) represent separate experiments. The data give the mean values ± SEM of a minimum of 4 incubations.

Piribedil has been found to be also ineffective, at a concentration up to 10^{-4} M, in another type of in vitro preparation, namely in homogenates of rat striatum[12]. In the same preparation, however, the piribedil metabolite S-584 was able to stimulate cyclic AMP production, with a maximal activation of 81% above baseline values[12]. This effect was blocked by 10^{-5} M chlorpromazine or 10^{-4} M spiroperidol[12]. Activation of adenylate cyclase by 10^{-5} M S-584 has also been recently observed in homogenates of rat caudate nucleus by other investigators[16]. These results suggest that the pharmacological effect of piribedil may be due to the formation in vivo of an active metabolite, such as the catechol compound S-584, which stimulates directly the dopamine-receptors of central nervous system.

A positive effect of S-584 on rat striatal homogenates and a negative effect of the same compound on isolated retina of the rabbit, as shown in the Figure, can be tentatively explained in terms of fundamental differences between the two models used for measuring dopamine- or dopamine-like adenylate cyclase activity. The maintenance of all membrane constituents, such as in the intact cells of the isolated tissue, might be of more physiological or pharmacological significance. Support for this hypothesis is provided by the fact that dopamine did not alter the concentration of cyclic AMP in slices from limbic forebrain[17], although a dopamine-sensitive adenylate cyclase exists in homogenates of mesolimbic structures and is inhibited by neuroleptics[2,3]. However, this lack of specificity probably does not occur when using rabbit retina, since, in both types of preparation, i.e. intact cells[5] or homogenates[6], dopamine as well as apomorphine were found to be very active. This provided a valid reason to investigate the effects of both piribedil and piribedil metabolite S-584 on homogenates of rabbit retina. As shown in the Table, neither drugs at 10^{-4} M concentration were able to stimulate the adenylate cyclase of homogenates of rabbit retina under conditions where the agonistic effect was maximal and well in accordance with our previous data[6]. The source of the discrepancies must therefore be looked for elsewhere.

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Since the caudate nucleus of the rat appears to contain both β-adrenergic- and dopamine-receptors[15], interaction of dopamine, "dopamine-like" drugs or dopamine antagonists with both types of receptors could occur. In line with this hypothesis is the recent finding of the blockade by the potent neuroleptics pimozide and clozapine of a noradrenaline sensitive adenylate cyclase in slices of rat limbic forebrain[17]. Furthermore, investigations in vivo show significant differences between the actions of apomorphine and piribedil, since the effects of piribedil are reduced by reserpine or tyrosine hydroxylase inhibition, whereas those of apomorphine are not[18,19]. It has also been suggested, on the basis of a variety of experimental data, that some endogenous release of dopamine must always exist for piribedil to exert its full dopamine receptor stimulating activity[20]. Thus the mechanism of action of piribedil remains to be elucidated. Whether or not it is related to noradrenergic mechanisms rather than dopaminergic mechanisms needs further investigation.

Summary. Using isolated retinas of the rabbit, we have shown that the effects of antiparkinsonian drugs such as piribedil and S-584 are not related to direct stimulation of dopamine-receptors.

M. SCHORDERET[21]

Département de Pharmacologie
Ecole de Médecine 20,
CH-1211 Genève 4 (Switzerland), 7 July 1975.

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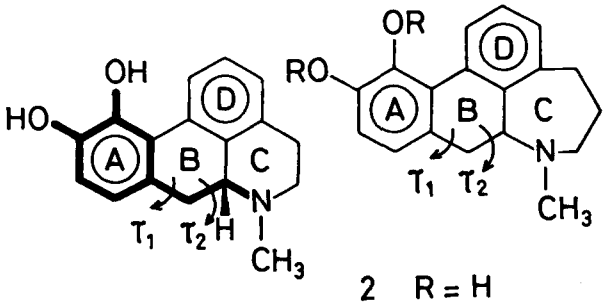
Conformations and Biological Properties of Apomorphine and its Phenanthro[10,1-b, c] azepine Homologue[1]

Most drug-receptor interactions are stereospecific and preferentially involve a particular conformation of the drug molecule. The stimulating effect of apomorphine (**1**) on dopamine receptors is very stereoselective. Only the (–)-enantiomer, which has the *R*-configuration[2], is biologically active[3,4]. This action may be attributed to that structural fragment of the apomorphine molecule which corresponds to dopamine. The conformation of this fragment has torsion angels in the $-CH_2-CH_2-N$ moiety which are fixed as anticlinal (τ_1) and antiplanar (τ_2) by the rest of the molecule.



1

2 R = H

3 R = CH_3

The values of τ_1, τ_2 observed in the crystal structure analysis of apomorphine hydrochloride[5] are 146, −178° and 133, −178° in the two independent molecules in the crystallographic asymmetric unit. These values contrast with those observed in the crystal structure of the flexible molecule dopamine in the form of its hydrochloride[6] where $\tau_1 = \pm 99°$, $\tau_2 = 180°$. In solution, however, the fractional population of this rotamer is only 0.43[7] and it is possible for dopamine to take up a conformation similar to that seen in apomorphine by rotation about the ring-C_α bond. Since, however, the conformation of the catecholamine moiety in apomorphine is different from the observed crystal conformation of dopamine, the relevance of the crystal conformation of dopamine to any arguments concerning dopamine receptors is called in question. The conformations of rigid analogues with high agonist activity are more likely to provide suitable indicators.

Recently, we have synthesized 11,12-dihydroxy-7-methyl-4,5,6,7,7a,8-hexahydrophenanthro[10,1-b,c]azepine (**2**)[8], which is a homologue of apomorphine (**1**). Contrary to expectation, this compound had no dopaminergic action. Oral doses up to 100 mg/kg had no effect on the general behaviour of mice, nor did 50 mg/kg i.p. affect the catalepsy induced by tetrabenazine. In rats, i.v. doses of between 0.5 and 20 mg/kg produced neither stereotypies nor gnawing. Apomorphine, when given under the same conditions, induced gnawing within 30 min, with an ED_{50} of about 0.3 mg/kg i.v.

There are two possible explanations for the inactivity of the apomorphine homologue **2**: either the nitrogen atom is not pseudoequatorial to ring B, as in apomorphine, but axial, and the molecule thus does not fit the receptor; or the nitrogen atom is equatorial and the receptor is unusually sterically sensitive in the region to which the C-ring of apomorphine binds. To distinguish between these two possibilities, we have completed a crystal structure analysis of the dimethoxy analogue **3**. NMR-spectra have shown that **2** and **3** have identical conformations.

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