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RETINAL TOXICITY AND TISSUE —SH LEVELS

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Abstract—Three substances, sodium iodoacetate, diphenylthiocarbazone (dithizone) and 1,5-di-(*p*-aminophenoxy)pentane dihydrochloride (M & B 968A), produce irreversible damage to the neuroepithelium of the rabbit retina when injected intravenously. These substances have been shown to produce a consistent elevation in "total —SH" levels in the retina and in contrast, produce a variable effect on "soluble —SH". Similar effects on —SH group levels were obtained with a fourth agent, sodium fluoride, given with potassium permanganate as adjuvant. The latter combination also produces a marked retinotoxicity. The possibility that the retinotoxic action of these agents is related to a specific protein denaturation effect is discussed.

FOUR substances—sodium iodate, sodium iodoacetate, the diabetogenic agent dithizone (diphenylthiocarbazone) and the schistosomicidal drug 1,5-di-(*p*-aminophenoxy)pentane dihydrochloride (M & B 968A) produce irreversible damage to the neuroepithelium of the rabbit retina after a single intravenous injection of an adequate dose,[1–4] whilst a fifth, sodium fluoride, provokes an inconsistent response, more fully discussed below.[5] These substances produce degenerative changes in the mammalian retina which follow almost identical ophthalmoscopic and histological patterns. The finding that cysteine and certain other aliphatic thiol compounds protected the retina against the toxic effects of iodate,[6] suggested the possibility that the retinotoxic effect is determined by an alteration in —SH levels in the retina. Sorsby and Reading[7] found a marked elevation in both "soluble" and "total —SH" within 16–20 hr after the injection of a retinotoxic dose of sodium iodate. The present investigations were undertaken to determine whether similar changes were produced by other retinotoxic substances.

EXPERIMENTAL

Male, 4-month-old Himalayan rabbits of the strain maintained at the Institute for Medical Research, Mill Hill, London, were used in all experiments. The rabbits were maintained under controlled conditions of diet and laboratory care for at least one week prior to experimentation and each animal was fasted 24 hr prior to medication. The substances were administered by a single intravenous injection as solutions in either physiological saline or distilled water at the following dosage levels; sodium iodoacetate 30 mg/kg, 1,5-di-(*p*-aminophenoxy)pentane dihydrochloride 5 mg/kg, dithizone 30 mg/kg, and sodium fluoride 50 mg/kg (sodium fluoride was given together with potassium permanganate 5 mg/kg as adjuvant).

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Rabbits were killed at the stated intervals and removal of the eyes and treatment of the retinae were carried out as previously described.[7] Most values recorded are based on six to eight estimations and in no case less than four. The potentiometric method of Calcutt and Doxey[8] used for the determination of —SH levels differentiates between "total —SH" and "soluble —SH". "Total —SH" levels represent —SH groups freely available within the tissue and capable of reacting directly with the specific titration reagent (*p*-chloromercuribenzoate) without any previous solubilization of tissue constituents by homogenization or precipitation, etc. "Soluble —SH" levels represent —SH groups present in substances soluble in 30% trichloroacetic acid extracts of tissue.

RESULTS

1. *Normal*

The mean value of thirty-four determinations on normal rabbit retinae were "total —SH" 17·2 (±0·8) and "soluble —SH" 10·5 (±1·5), both expressed as μg —SH/100 mg (wet wt.) tissue. Numbers in parentheses are the standard errors of the means (S.E.M.).

2. 1,5-*di*-(p-*aminophenoxy*)*pentane dihydrochloride* (*Fig.* 1a)

1,5-di-(*p*-aminophenoxy)pentane dihydrochloride produced a slight fall in both "soluble" and "total —SH" levels 30 min after injection, but within 1 hr both levels had risen markedly, the rise being maintained up to 8 hr after injection and remaining steady up to 18 hr. Seven days after injection —SH levels returned to normal.

3. *Sodium iodoacetate* (*Fig.* 1b)

Although the effect of sodium iodoacetate on retinal "total —SH" levels was less marked than that of the other retinotoxic agents, an immediate rise in "total —SH" was recorded, followed by a return to the normal level within 12 hr. Thereafter a slow but steady fall in "total —SH "levels was found. "Soluble —SH "levels fell rapidly after iodoacetate administration and remained at a low level up to 12 hr, followed by a return towards normal values.

4. *Dithizone* (*Fig.* 1c)

Dithizone completely abolished "soluble —SH" levels up to 48 hr after injection, but like the other retinotoxic agents (after initial fluctuation) increased "total —SH" values within 2 hr. The elevation in "total —SH" although not particularly striking, was more prolonged with this compound; even 48 hr after injection an increase of about 15 per cent above normal was recorded.

5. *Sodium fluoride and potassium permanganate* (*Fig.* 1d)

Intravenous injection of sodium fluoride in the maximum tolerated dose (50 mg/kg) produces a retinotoxic response in some 20 per cent of rabbits treated, but the incidence of lesions rises to some 70 per cent if the animals are fasted for 24 hr prior to injection.[9] Preliminary results on animals so fasted and treated showed a very variable response on retinal —SH levels; the only consistent value obtained was 1 hr after injection when the "total —SH" level was increased to 23 μg/100 mg (wet wt.) tissue, an increase of about 24 per cent above the normal. Sorsby and Harding,[10] showed that potassium

permanganate and certain other oxidizing agents, which themselves are not retinotoxic, potentiate the effect of some of the retinotoxic compounds, especially sodium fluoride. Injection of potassium permanganate alone (5 mg/kg) produced a marked drop in "total —SH" level; 1 hr after injection the value of "total —SH" was 8·5 μg/100 mg (wet wt.) tissue (a decrease of about 51 per cent from normal), whilst "soluble —SH" was negligible.

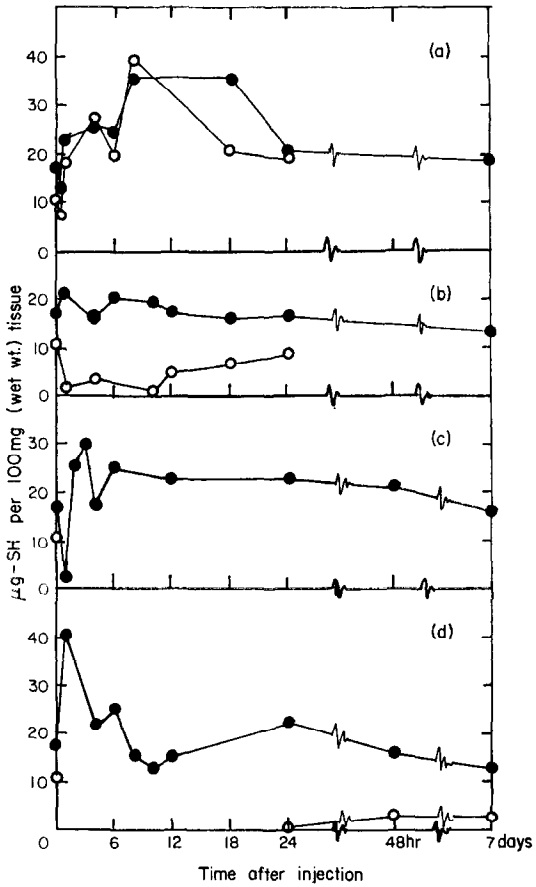

FIG. 1. —SH levels in rabbit retinal tissue following a single intravenous injection of:
(a) 1,5-di-(*p*-aminophenoxy)pentane dihydrochloride, 5 mg/kg;
(b) sodium iodoacetate 30 mg/kg;
(c) diphenylthiocarbazone (dithizone), 30 mg/kg and
(d) sodium fluoride 50 mg/kg, together with potassium permanganate 5 mg/kg, as adjuvant.
"Total —SH" levels—●; "Soluble —SH" levels—○.

Sodium fluoride (50 mg/kg) together with potassium permanagnate (5 mg/kg) produced a striking and very rapid rise in "total —SH" levels, exhibiting a maximum 1 hr after injection. Thereupon "total —SH" values fell below normal within 8–12 hr, rising and falling again until 7 days after injection when the value was below normal. "Soluble —SH" values were abolished altogether and were negligible up to 24 hr

after injection, whereupon this value was just estimable, but remained at a low level at 48 hr and 7 days (about 25 per cent of the normal).

DISCUSSION

The basis of the retinotoxic effect

At first sight, the five retinotoxic substances appear to have little in common either chemically or pharmacologically. However, they all produce marked degeneration of the visual cells of the mammalian retina and the present experiments have shown that they all produce an increase in retinal "total —SH" values. The rise in "total —SH" levels is consistent with the idea of retinal protein denaturation, thereby "unmasking" —SH groups previously inaccessible to chemical reaction. The variability of the "soluble —SH" levels fits in with the idea of increased lability of retinal protein. Bearing on this the following observations are relevant: (a) following the injection of 1,5-di-(*p*-aminophenoxy)pentane dihydrochloride, the levels of "soluble —SH" exceeded those for "total —SH" at 8–12 hr. This apparent anomaly is thought to be a consequence of the retinotoxic compound exerting a denaturing effect on retinal protein so that the latter is rendered unstable. Under such conditions, the operation of grinding the tissue in 30% trichloracetic acid is probably drastic enough to "unmask" further —SH groups as soluble constituents. A similar phenomenon was observed following the injection of sodium iodate.[7] (b) The results of the experiments with fluoride used in conjunction with potassium permanganate are of especial interest. Permanganate alone reduced the "total —SH" level, whereas when given as an adjuvant to fluoride, the result was a marked and very rapid rise in "total —SH" levels accompanied by an acute retinotoxic effect. Since potassium permangantte by itself is not retinotoxic,[10] it is unlikely that retinotoxicity of this type is related to an overall decrease in tissue —SH.

Rôle of thiol groups in the retina and their relation to retinotoxicity

The rôle of thiol groups in the retina is still subject to speculation, but Wald and his colleagues,[11] have shown that the action of light in the bleaching of rhodopsin in the vertebrate retina, is associated with the "unmasking" of —SH groups; two per molecule of visual pigment. Although thiol groups are not involved in the direct chemical combination between 11-*cis* retinene and opsin, they may be involved in the conformation of the protein moeity by hydrogen bonding. It is therefore possible that the retinotoxic substances exert an effect on the retina which amounts to an irreversible bleaching with subsequent structural changes.

Cysteine and certain other aliphatic thiol compounds, when injected at the same time as the toxic agent, protect the retina from the effects of iodate only, and not from the effects of the four other agents mentioned.[6] Since cysteine and iodate react readily in the test-tube, it seems likely that the protective action of the former is due to chemical reaction occurring systematically, so that an effective concentration of iodate is not attained in the retina. This observation provides additional indirect evidence that the primary focus of action of the retinotoxic substances does not lie in chemical reaction at the target site, with soluble, low molecular weight thiol compounds.

Although the increase in tissue —SH as a manifestation of protein denaturation offers a reasonable explanation for breakdown in retinal function, it is difficult to carry this explanation further to account for the irreparable structural damage to the

neuro-epithelium of the retina which ensues, unless one assumes that cellular breakdown is a secondary effect following specific retinal protein denaturation. In support of such an assumption, there is the well established fact that the integrity of membrane —SH groups bears a direct relation to permeability characteristics.[12] Since the outer segment membrane structure of the visual cells is early affected in retinotoxicity, it is not difficult to envisage a breakdown in cellular structure due to changes in membrane permeability.

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