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Inhibition of Axoplasmic Transport by Mescaline and Other Trimethoxyphenylalkylamines

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SUMMARY

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Following an intraocular injection of [^{3}H]L-proline, fast axoplasmic transport can be demonstrated in the rat optic system by a flow of ^{3}H-labeled protein which moves from the ganglion cells of the retina at a rate of approximately 16 mm/hr. The injection of mescaline or one of several trimethoxyphenylalkylamines inhibits the transport of labeled proteins through the optic nerve. By using one eyeball containing the drug as the experimental eye and the contralateral eye as a control, the degree of inhibition may be determined quantitatively, allowing evaluation of dose-response curves. Dose-response curves for the inhibition of axoplasmic transport by mescaline, 1-(3,4,5-trimethoxyphenyl)-2-aminopropane, and 1-(2,4,5-trimethoxyphenyl)-2-aminopropane have been obtained. Further experiments with mescaline indicate that (a) the inhibition of fast axoplasmic transport produced by mescaline in the rat optic system is reversible, (b) mescaline inhibits transport when applied directly to the cat sciatic nerve, and (c) mixtures of component pieces of the molecular skeleton of mescaline, trimethoxybenzene and ethylamine, have little activity as inhibitors of fast axoplasmic transport in either the rat optic or cat sciatic nerves. Several of the agents found effective as inhibitors of axoplasmic transport are known hallucinogens. The hallucinogenic potencies of these drugs rank in the same order as their effectiveness as antitransport agents.

INTRODUCTION

Axoplasmic transport can be defined as the active movement of materials from the nerve cell body, or perikaryon, through the axon toward the synaptic terminal. Among the materials carried by axoplasmic transport are macromolecules which are assembled in the perikaryon, including proteins (1, 2), glycoproteins (3, 4), and small amounts of RNA (5, 6). The importance of transported materials for synaptic function has recently been demonstrated experimentally (7, 8).

Little is known about the mechanism of axoplasmic transport. At least two rates of transport are observed in most systems: a slow flow of approximately 1–3 mm/day (9–11), and a faster rate ranging from about 100 to 400 mm/day (1, 2, 12–14). Because of the relatively short time scale required to study the faster rates, fast axoplasmic trans-

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port is the better characterized. Fast axoplasmic transport is an active process which derives its energy locally along the nerve, and is blocked by various metabolic inhibitors such as iodoacetate and dinitrophenol (15, 16). Inhibition of axoplasmic transport is also observed with two classes of antimitotic drugs, typified by colchicine (17–19) and vinblastine (20–22). These drugs are thought to inhibit cell division through disruption of the microtubules of the mitotic spindle (23, 24). Isolated microtubule subunits, designated as tubulin or microtubule protein, bind colchicine and vinblastine at different sites (25). Inhibition of axoplasmic transport by colchicine and vinblastine has been cited (17–19, 26) as support for the hypothesis that the microtubules which are always present in axons are involved in the transport process.

In the past many biological processes have been clarified using inhibitory drugs. We believe that extension of the list of agents which inhibit axoplasmic transport may facilitate investigations into the mechanism and function of transport. A rapid method of assessing and quantitating the effect of drugs on fast axoplasmic transport has been described (27, 28). Using a modification of this method, we have been screening drugs for inhibition of the transport process. Through examination of the structures of colchicine derivatives which are both active and inactive as inhibitors of axoplasmic transport (27, 28), the trimethoxyphenylalkylamines were chosen as smaller molecules which might retain the antitransport activity of colchicine. Several drugs in this class, including mescaline and various analogues, have now been tested and found to be effective inhibitors of fast axoplasmic transport.

METHODS

Two systems were used to study the effects of drugs on axoplasmic transport. Both systems involved incorporation of [^{3}H]L-proline into proteins of the nerve cell bodies and the subsequent transport of ^{3}H-labeled proteins down the nerve. Inhibition of axoplasmic transport was inferred from a measurable interference in the transport of labeled materials through the nerve. Since different criteria for inhibition were used in the two experimental systems, these will be discussed separately.

The rat optic system provided the more convenient method of the two. The nerve cell bodies of the optic nerve reside in the ganglion cell layer of the retina, which was exposed to either labeled precursors or drugs by injection into the posterior chamber of the eyeball (27, 29). If a [^{3}H]amino acid was included in the injection, it was incorporated into retinal protein which was transported down the optic nerve at approximately 16 mm/hr and accumulated at the two main fiber terminals in the brain, the superior colliculus and the lateral geniculate body (30).

In a typical experiment a 225-g female rat was anesthetized with sodium thiopental (15 mg intraperitoneally). Into one eye (the experimental eye) were injected 10 μl of 0.9% NaCl containing both the drug of interest and 45 μCi of [2,3-^{3}H]L-proline. The contralateral eye (the control eye) was treated with a similar solution lacking the drug. The animal was killed after approximately 3 hr. The optic nerves were removed, cut into 1-mm sections, and solubilized in Soluene-100 by incubation overnight at room temperature; this solution was diluted with scintillation fluid prior to counting in a Beckman LS-230 liquid scintillation spectrometer. Incorporation of [^{3}H]L-proline into retinal protein was determined by homogenizing the previously weighed retina in 18% trichloracetic acid, dissolving the residue in Soluene-100, and counting an aliquot of the resulting solution. When a drug was insoluble in 0.9% NaCl, a vehicle containing equal volumes of 0.9% NaCl and dimethylformamide was employed. Control experiments showed that injection of 10 μl of dimethylformamide had no effect on either axoplasmic transport or the incorporation of [^{3}H]L-proline into retinal protein. Inhibition of transport by a drug was detected by a decreased level of transported radioactivity in the experimental nerve with respect to the control nerve.

A second procedure proved useful when it was desired to expose only the axons of neurons to the action of a drug. In this case

experiments were conducted with the sciatic nerves of cats, using the surgical procedures of Ochs and Burger (31) and Lasek (32). Adult animals (2.5–3.5 kg) were immobilized on a stereotaxic apparatus (33) after exposure of both dorsal root ganglia. Into each ganglion were injected 2.5 μl of 0.9% NaCl containing 20 μCi of [^{3}H]L-proline. With the aid of a mechanically driven micro-syringe of our design, each injection was made over a period of 10 min at a depth of 800 μ through glass micropipettes of about 20-μ diameter at the tip. Upon completion of the injections, the wound was cleaned and sutured. Immediately afterward both sciatic nerves were exposed by making small incisions in the skin at the hip joint at a location just distal to the branch point of the gluteus nerves from the main sciatic nerve trunk. Using a 100-μl Hamilton microsyringe, the drug was injected just under the epineurium of one sciatic nerve. Drugs which were not sufficiently soluble in aqueous solutions were dissolved in a 1:1 mixture of 0.9% NaCl and dimethylformamide, of which 25 μl were then injected. An appropriate control injection was placed in the other sciatic nerve of each animal. The incision was closed with a single suture, and the animal was placed on a heating pad and allowed to recover. After 24 hr the cat was killed and the sciatic nerves were dissected free. The nerves were then trimmed of small adventitious branches, cut into 3-mm pieces, and solubilized in Soluene-100 (room temperature, 24 hr); the resulting solution was diluted with scintillation fluid before counting. The effect of a drug upon transport was indicated by an accumulation of transported radioactivity in the nerve sections proximal to the site of injection.

Materials. Female rats of the NLR strain were procured from National Laboratories, St. Louis. Sodium thiopental was purchased from Abbott Laboratories. [2,3-^{3}H]L-Proline HCl was obtained from New England Nuclear Corporation at a specific activity of 35 Ci/mmole and a concentration of 10 mCi/ml. The tissue solubilizer, Soluene-100, was purchased from Packard Instrument Company. To prevent chemiluminescence from tissue samples dissolved in Soluene-100, a scintillation mixture containing 9.15 g of benzoic acid and 9.0 g of 2,5-diphenyloxazole in 3.0 liters of toluene was employed. Mescaline, 1,2,3-trimethoxybenzene, and ethylamine hydrochloride were obtained from Aldrich Chemical Company. The hydrochlorides of 1 - (3,5 - dimethyoxy - 4 - hydroxyphenyl) - 2-aminoethane and 1-(3,5-dimethoxy-4-hydroxyphenyl) - 2 - (N,N - dimethylamino)-ethane, as well as the hydrobramide of 1 (2,6 - dibromo - 3,4,5 - trimethoxyphenyl) - aminoethane, were generously donated by Hoffmann–La Roche, Inc.

RESULTS AND ANALYSIS

Quantitation of inhibition of fast axoplasmic transport. In the rat optic system an estimate of the effect of a drug on transport may be obtained by comparing the level of radioactivity found in the experimental nerve with that found in the control nerve (27, 28). In some cases, however, drugs or experimental techniques cause differential incorporation of ^{3}H-labeled precursor from eye to eye. It is possible to correct for such differences of incorporation, since the appearance of radioactivity in the nerve is directly proportional to the amount of labeled precursor incorporated into retinal protein (see below). To carry out this correction, we have defined three parameters. The transport ratio (t_i) compares the amount of tritiated material found in the experimental and control nerves:

$$t_i = \frac{(\text{cpm/mm experimental nerve})_i}{(\text{cpm/mm control nerve})_i}$$

A t_i value is obtained for each of the i pairs of 1-mm sections taken at matched distances from the eyeball. Sectioning error is reduced by calculating the mean (T) and standard deviation for the values of t_i. To utilize the correction which can be obtained from the amount of [^{3}H]precursor incorporated into the experimental and control retinas, a protein incorporation ratio (P) is defined, in much the same way as t_i:

$$P = \frac{\text{cpm in experimental TCA residue/mg experimental retina}}{\text{cpm in control TCA residue/mg control retina}}$$

where "TCA residue" refers to the insoluble residue of the retinal tissue homogenized

in trichloracetic acid. With no depression of transport in the experimental nerve, T should equal P. If, however, a drug depresses transport without affecting incorporation of the precursor, T will be smaller than P. As a corrected measure of transport in the experimental nerve we have defined a net transport ratio (NT):

$$NT = \frac{T}{P}$$

An NT value of 1.00 would indicate no net depression of transport, while a value of 0.00 would indicate complete blockage. Intermediate values of NT would correspond to partial blockage.

To test the validity of the corrections involved in the NT parameter, we examined data from a series of animals in which both eyes were treated with solutions containing [^{3}H]L-proline but lacking any drug ("no drug" controls). We also considered animals which received doses of mescaline which had no effect upon transport (25 μg), and animals in which a purposeful imbalance was achieved by injecting different amounts of precursor into the two eyes. Since in all these animals transport should have been unaffected, the predicted value of NT was 1.0. The average experimental value of NT was 1.01 ± 0.05 (SD, $N = 15$). As T values for these animals ranged from 0.03 to 1.74, the NT function provides a satisfactory correction for differential incorporation of [^{3}H]L-proline into retinal protein.

In this system it is possible for the radioactive precursor to generalize from one eye through the bloodstream to the contralateral nerve. Using a control in which [^{3}H]L-proline was injected into only one eye, with 0.9% NaCl in the other, the amount of radioactivity found in the optic nerve due to generalization was less than 2% of that found in the control nerve.

To test the response of the parameters defined to this point, a number of drugs were examined in preliminary experiments. In all cases in which the drug affected transport, the values of t_i decreased from the highest value, in the section attached to the eye, through intermediate values in the next 3 mm, to a plateau value which was found in all sections 5 mm or more from the eye. The value of t_i in the plateau region was related to the dose of the drug employed (see DISCUSSION), while the values of t_i at points nearer the eye were not. In order to avoid systematic errors which would be introduced into the data by this phenomenon, only t_i values from sections of nerve at distances greater than 4 mm from the eye were used to calculate values of T for the remaining experiments presented in this paper.

With the caution expressed in the last paragraph, the NT parameter should provide a quantitative measure of the effect of a drug on transport. To test the response of this parameter to drugs known to inhibit axoplasmic transport, 8 μg of vinblastine were introduced into the experimental eye of one animal, and 0.5 mg of colchicine into the experimental eye of another. The resulting NT values were 0.19 and 0.41, respectively, in good agreement with previous quantitative data (27, 28). To determine the sensitivity of the NT parameter to a drug known to inhibit protein synthesis, cycloheximide at doses ranging from 10 to 250 μg was included in the experimental injection. All doses depressed the incorporation of [^{3}H]L-proline into trichloracetic acid-insoluble retinal proteins to approximately 10% of the incorporation seen in the control retina. NT values in these experiments were approximately 1.0 or greater, indicating no apparent inhibition of transport.

Effects of mescaline and other trimethoxyphenylalkylamines on fast axoplasmic transport. Several trimethoxyphenylalkylamines were examined for effectiveness as inhibitors of fast axoplasmic transport in the rat optic system. Specific compounds and the observed depression of transported material are presented in Table 1.[2]

Using the NT parameter as a continuous scale of the inhibition of transport, it is possible to evaluate dose-response curves for compounds of interest. Dose-response curves have been evaluated for 1-(3,4,5-trimethoxyphenyl)-2-aminoethane (mescaline), 1-(3,4,5 - trimethoxyphenyl) - 2 - aminopropane (I), and 1-(2,4,5-trimethoxyphenyl)-2-aminopropane (II) (Fig. 1). In this series of three drugs compound II is a more effective inhibitor of axoplasmic transport than I, which in turn is more effective than mescaline.

[2] Compound I is 1-(3,4,5-trimethoxyphenyl)-2-aminopropane; II, 1-(2,4,5-trimethoxyphenyl)-2-aminopropane; III, 1-(3,4,5-trimethoxyphenyl)-2-aminobutane; IV, 1-(3,5-dimethoxy-4-hydroxyphenyl)-2-aminoethane; VI, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane; VII, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminobutane. In some of the literature referenced in this paper compound I is referred to by the abbreviation TMA, II by TMA-2, and VI by DOM.

TABLE 1

Effect of structural analogue of mescaline upon axoplasmic transport from the rat eye
Details of the measurements are discussed under METHODS.

Agent	Dose	Net transport (NT)
	mg	
1-(3,4,5-Trimethoxyphenyl)-2-aminoethane HCl (mescaline)	0.40	0.50[a]
1-(3,4,5-Trimethoxyphenyl)-2-aminopropane HCl (I)	0.32	0.50[a]
1-(2,4,5-Trimethoxyphenyl)-2-aminopropane HCl (II)	0.17	0.50[a]
1-(3,4,5-Trimethoxyphenyl)-2-aminobutane HCl (III)	0.5	0.87
	1.0	0.63
1-(3,5-Dimethoxy-4-hydroxyphenyl)-2-aminoethane HCl (IV)	0.5	0.58
	1.0	0.09
1-(3,5-Dimethoxy-4-hydroxyphenyl)-2-(N,N-dimethylamino)ethane HCl (V)	0.5	0.59
	0.5	0.65
	1.0	0.42
1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane HCl (VI)	0.10	0.30
	0.50	0.07
1-(2,5-Dimethoxy-4-methylphenyl)-2-aminobutane HCl (VII)	0.27	0.54
	0.64	0.16
1-(2,6-Dibromo-3,4,5-trimethoxyphenyl)-2-aminoethane HBr (VIII)	0.1	0 81
	0.62	0 99

[a] Interpolated from the data of Fig. 1.

Although not shown in the data of Table 1 or Fig. 1, the drugs tested in this study can affect the incorporation of precursors into proteins of the retina if sufficiently high doses are employed. For example, doses of mescaline in excess of 1.0 mg reduced the P parameter, although lower doses produced no consistent change from the expected value of 1.0. Compounds I and II were less effective in reducing incorporation. These agents produced a measurable reduction in the P parameter only at the highest doses tested (1.5 mg).

In order to see whether the entire molecular skeleton of mescaline was needed for an effect upon transport, an experiment was performed in which the experimental injection contained 1,2,3-trimethoxybenzene and ethylamine hydrochloride, each at molar levels equivalent to 1 mg of mescaline. In this experiment depression of neither transport ($NT = 0.98$) nor incorporation of precursor into retinal proteins was seen. "Breaking" the molecule of mescaline between the alkyl chain and the aromatic ring effectively removes the antitransport activity.

Effect of mescaline on axoplasmic transport in cat sciatic nerve. All experiments done in the rat optic system involve exposing the nerve cell bodies to a fluid containing the drug being studied. In this system transport could be blocked through interaction of a drug with some step intermediate between the synthesis of protein and the commitment of protein to the transport process. To determine whether these drugs would interfere with the transport of protein previously committed to the transport process, several experiments were conducted in which drugs were applied directly to sensory axons of the cat sciatic nerve. As described under METHODS, injections of 25 μl of a vehicle with or without a drug were introduced under the epineurium of the sciatic nerve after previous

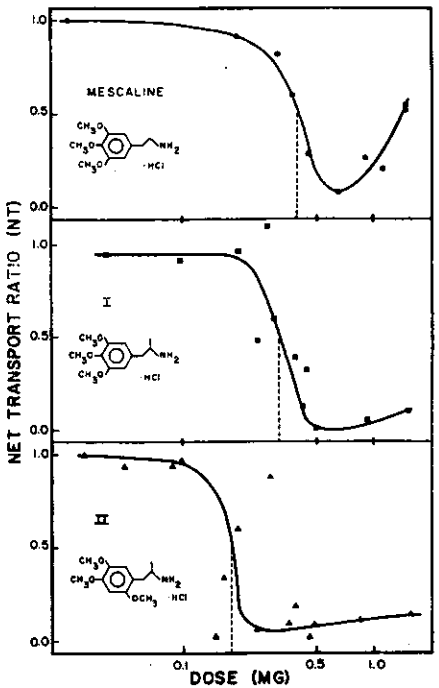

FIG. 1. *Dose-response curves for inhibition of fast axoplasmic transport by mescaline (top frame), compound I (middle frame), and compound II (bottom frame), using the NT parameter as net measure of transport in experimental nerve*

Dashed lines intersect each curve at an *NT* value of 0.50 to indicate the relative dose of each drug required to produce 50% inhibition of fast axoplasmic flow. These lines correspond to a dose of 0.40 mg for mescaline, 0.32 mg for I, and 0.17 mg for II. For a discussion of the *NT* parameter, see the text.

injections of [^{3}H]L-proline had been made into the L_7 dorsal root ganglia. During the course of the experiment the [^{3}H]L-proline is incorporated into protein in the ganglion cells and transported down the sciatic nerve at a rate of approximately 400 mm/day (34, 30).

The criterion used for blockade of transport was the accumulation of transported radioactivity at the site of injection of the drug, on the side nearer the ganglion. No accumulation of radioactivity was seen when injected solutions contained either 0.9% NaCl or a solution composed of equal volumes of dimethylformamide and 0.9% NaCl. Injection of 0.1 mg of mescaline in 0.9% NaCl also failed to produce accumulation. However, large accumulations of radioactivity were observed in two identical experiments when higher doses of mescaline (5.0 mg) were injected, using a vehicle of equal volumes of dimethylformamide and 0.9% NaCl (Fig. 2). The contralateral (control) nerve of these animals received an injection of a solution containing 1,2,3-trimethoxybenzene and ethylamine HCl, each in molar amounts equal to 5.0 mg of mescaline HCl. The amount of radioactive material accumulated in each control nerve was 10–20% of that found in the experimental nerves. Mescaline exhibits an antitransport activity when applied directly to nerve axons. This activity cannot be accounted for by a similar

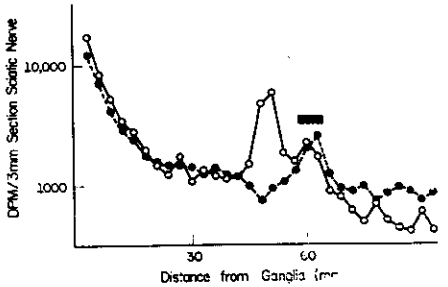

FIG. 2. *Effect of mescaline on axoplasmic transport by sensory neurons of cat sciatic nerve*

Injections of [^{3}H]L-proline were made into the L_7 dorsal root ganglia of an adult cat (see METHODS). The desired drug was injected under the epineurium of the sciatic nerve approximately 50 mm from the ganglia. Injections were made at the center of the length indicated by the horizontal bar. Injections of dyes indicated that the extent of diffusion of the injected material was about the length indicated by the horizontal bar. ○——○, transported radioactivity in the experimental nerve, which was treated with 5.0 mg of mescaline. ●– – –●, transported radioactivity in the contralateral nerve, which was treated with an equimolar mixture of 1,2,3-trimethoxybenzene and ethylamine HCl, each in molar amounts equivalent to 5.0 mg of mescaline. Accumulation of transported material was inferred from the radioactivity in the peaks located proximal to the site of injection and above a baseline established for each nerve by connecting the plateau levels of radioactivity before and after the peaks of accumulation.

dose of its component pieces, trimethoxybenzene and ethylamine.

Reversibility of inhibition of axoplasmic transport by mescaline. By shifting the time scale of the experiments in the rat optic system, we were able to demonstrate that the inhibition of transport produced by mescaline was reversible. Mescaline (1.0 mg in 10 μl of 0.9% NaCl) was injected into one eyeball of a rat, using as a control in the other eye a similar injection without mescaline. At various times after these injections [^{3}H]L-proline in 0.9% NaCl was introduced into both eyes. Three hours after injection of the [^{3}H]L-proline the rats were killed and carried through the procedure described under METHODS. Inhibition of transport was still quite marked 24 and 48 hr after the injection of mescaline, when net transport values (*NT*) of 0.11 and 0.18, respectively, were observed. At 96 hr, however, the *NT* value had increased to 0.97, indicating that the inhibition of transport had been completely reversed.[3]

DISCUSSION

Several antimitotic drugs of the class typified by colchicine are effective inhibitors of fast axoplasmic transport (17–19, 27). Examination of the structures of active compounds, such as colchicine, colchiceine, *N*-deacetyl-*N*-methylcolchicine (Colcemid), and podophyllotoxin, as well as inactive derivatives, such as colchicoside and picropodophyllin, suggested to us that smaller molecules such as the trimethoxyphenylalkylamines might also inhibit transport. In support of this hypothesis, mescaline was found to interfere with the transport process in the rat optic and cat sciatic nerves. In addition, several structural analogues of mescaline have varying degrees of effectiveness as inhibitors of axoplasmic transport in the rat optic system. Colchicine is thought to act by binding to the subunit proteins of microtubules to disrupt the filamentous structure (35). Inhibition of axoplasmic transport by colchicine has provided support for the hypothesis that microtubules are involved in the transport process. If the mechanism of action of the antitransport activity of the trimethoxyphenylalkylamines is similar to that of colchicine, it is possible that these new agents inhibit fast axoplasmic transport through an interaction with microtubule protein. The fact that peripherally placed mescaline can inhibit transport in the cat sciatic nerve supports this suggestion. Experiments are currently under way to test the hypothesis directly.

The data collected in this study were obtained primarily from an experimental system based upon the optic pathway of the rat. Experiments done in the rat optic system to determine the effect of drugs on axoplasmic transport have the advantage of being both quick and quantitative. The results obtained here, however, point up two factors which must be considered when using the system. First, there exists an experimental problem in the 4 mm of nerve adjacent to the eye. While the appearance of radioactive material in the nerve sections further than 4 mm from the eye may be completely depressed by sufficient levels of a drug such as colchicine, a component of radioactive material is found in the first 4 mm regardless of the dose of the drug employed. When transport in the experimental nerve is most severely inhibited by colchicine, radioactive material in the first four 1-mm sections from the eye decreases exponentially to background levels which are observed in the remaining sections of the nerve nearer the optic chiasma. This "extra" component of radioactivity also becomes apparent in the t_i values of an experiment in which transport is depressed to a lesser extent by a drug in the colchicine or trimethoxyphenylalkylamine classes. In such a case the calculated values of t_i are not constant across the nerve, as is the case with "no-drug" controls, but instead decrease in the first 4 mm from the eye before reaching a plateau value in the remainder of the nerve. Because of these observations we have chosen to neglect the first 4 mm of the nerve. This procedure seems justified in view of the fact that the t_i values from these sections yield no data which are dose-related, while the plateau of t_i values seen in the remaining sections is clearly related to the levels of drug employed.

A second point concerning the experimen-

[3] Similar experiments using 1 mg of colchicine in five rats were not completed because of the death of all animals, presumably as a result of the colchicine.

tal system has to do with the use of the specific activities of the experimental and control retinas as corrective factors for incorporation of precursor. This practice was introduced to correct for variations in the amount of precursor injected into each eye. The technique should also serve to correct for imbalances produced by the presence of the drug. As examples, the drug could actually inhibit some aspect either of uptake of the precursor into the retina, or of the synthesis of protein. Of perhaps greater concern with these agents, many of which are sympathomimetic, is an alteration of the normal vascular drainage of one eye, thereby resulting in a change in the amount of available precursor. The correction procedure seems well supported in control experiments, in which a purposeful imbalance of labeled precursor was achieved. The validity of the correction in the presence of a drug which may affect transport is not, however, established by experiment. In such a case it seems most reasonable to use the procedure as a first-order correction, realizing that subtle errors may arise in specific instances. Such a second-order effect may be responsible for the slight increase in the NT parameter seen with mescaline at doses in excess of 1.0 mg. In fact, the observed increase is a result of overcorrecting T with P; the calculated T values decrease smoothly to nearly zero at doses of 1.0 mg or greater. At these doses, however, mescaline also strongly depresses the incorporation of precursor. This situation results in a calculated NT value which is a ratio of two small numbers, and hence is susceptible not only to experimental error but also to secondary metabolic effects. Compounds I and II, both of which exert much less effect upon incorporation than does mescaline, have correspondingly less increase in NT at high doses.

Finally, it should be pointed out that the corrections involved in calculating the NT parameter cannot correct for variation in the uptake of a drug by the retina. In practice this means that a reliable quantitation of the effect of a drug must be carried out by some means such as constructing a dose-response curve or duplicating experiments to ensure reproducibility. While this paper largely presents the former alternative, both appear promising.

Using the NT parameter as a measure of the inhibition of transport for a given dose of a drug, several structure-activity relationships can be drawn from the data of Table 1. The series I > mescaline > III varies only in the substituent on the α-carbon of mescaline. The substitution of a methyl group at this position, as in I, yields maximal activity. Substitution of an ethyl group, as in III, produced a compound which is less active than mescaline itself. The decrease in activity from the methyl to ethyl substitution is also seen with the structural analogues 1 - (2,5 - dimethoxy - 4 - methylphenyl) - 2 - aminopropane (VI) and 1 - (2,5 - dimethoxy-4 - methylphenyl) - 2 - aminobutane (VII). Replacing the p-methoxyl group of mescaline with a hydroxyl group by conversion of mescaline to 1-(3,5-dimethoxy-4-hydroxyphenyl)-2-aminoethane (IV) has little effect on activity. To assess the importance of the intact molecular skeleton of mescaline, equimolar mixtures of 1,2,3-trimethoxybenzene and ethylamine were tested in both the rat optic and cat sciatic systems. High doses of this mixture produced no detectable activity in the rat optic system and only 10–20 % of the activity in the cat sciatic nerve. Apparently the intact molecule is more active than the two pieces.

Several of the trimethoxyphenylalkylamines found to inhibit axoplasmic transport are known hallucinogens. Relative hallucinogenic potencies in man have been determined for mescaline (1.0), I (2.2), II (17), and VI (80) (36, 37). In several other mammals, including the squirrel monkey (38) and hooded rat (39), characteristic behavioral changes induced by hallucinogenic drugs have been described. Drugs may be ranked in order of their potency by the dose required to elicit these behavioral changes. In both the squirrel monkey and the hooded rat, II is more effective than I, which was more effective than mescaline (29). These three drugs have the same order of effectiveness as inhibitors of axoplasmic transport (II > I $\gtrsim$ mescaline; Fig. 1). In addition, III, which is less effective than mescaline as an inhibitor of axoplasmic transport, is inactive as a hallucinogen in man at dose levels greater than those required to produce a psychotomimetic episode with I (40). While qualitative comparisons show a correlation between the effectiveness of some of these drugs as hallucinogens and as inhibitors of axoplasmic transport, quantitative comparisons are not as easily interpreted. For example, VI is 5 times more potent than II as a hallucinogen in man, and is just slightly more effective than II in inhibiting axoplasmic transport. Using the dose required to inhibit axoplasmic transport by 50 %, II is 2.4 times more effective than mescaline (Table 1). While in man compound II is 17 times more potent a hallucinogen than mescaline, it is only 3.4 times more potent than mescaline in producing behavioral changes in the squirrel monkey (38). Even though these comparisons are made between species and using various criteria of effectiveness, a correlation seems to exist between the relative effectiveness of several trimethoxyphenylalkylamines as hallucinogens and as inhibitors of fast axoplasmic transport. It is possible that the two effects may be related.

Our purpose at the outset of these investigations was to test for drugs which would inhibit fast axoplasmic transport. Several trimethoxyphenylalkylamines have been found which exhibit this activity. Mescaline, representative of these drugs, is an inhibitor of axoplasmic transport in the cat sciatic nerve as well as a reversible inhibitor of axoplasmic transport in the rat optic nerve. Mescaline was also found to be much less toxic than colchicine,[3] which has approximately the same antitransport activity in the rat optic system. The trimethoxyphenylalkylamines further extend the list of drugs known to inhibit fast axoplasmic transport. It is hoped that with a selection of such drugs as tools, progress may be made in our understanding of the biological function and mechanism of axoplasmic transport.

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Structure-Activity Relationships of Cardiotonic Steroids for the Inhibition of Sodium- and Potassium-Dependent Adenosine Triphosphatase

I. Dissociation Rate Constants of Various Enzyme–Cardiac Glycoside Complexes Formed in the Presence of Magnesium and Phosphate

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SUMMARY

YODA, ATSUNOBU: Structure-activity relationships of cardiotonic steroids for the inhibition of sodium- and potassium-dependent adenosine triphosphatase. I. Dissociation rate constants of various enzyme–cardiac glycoside complexes formed in the presence of magnesium and phosphate. *Mol. Pharmacol.* 9, 51–60 (1973).

The dissociation rate constants (k_d) of cardiac monoglycoside–($Na^+ + K^+$)-ATPase complexes in the presence of magnesium and inorganic phosphate were determined by enzymatic assay after dilution. Among various cardiac monoglycosides, k_d was dependent on the nature of the sugar and the temperature but not on the steroid. The 3′-hydroxyl and the 5′-α-methyl group of the sugar markedly influenced the stability of the complex. The order of stability of cardiac monoglycoside–($Na^+ + K^+$)-ATPase complexes is the following: L-rhamnoside > D-6-deoxyguloside ≒ D-digitoxide > D-fucoside ≒ D-6-deoxyglucoside. Methylation or acetylation of the 3′-hydroxyl group decreased this stability. These data indicate a sugar site on the enzyme, and they suggest that the 3′-hydroxyl is bound to two groups at this site, a proton-donating group and a proton-accepting group. The 3′-α-hydroxyl binds to either group, but the 3′-β-hydroxyl and the 3′-α-methoxyl bind only a proton-accepting group and a proton-donating group, respectively. The activation energy of this dissociation was rather constant (20 kcal/mole) with various cardiac monoglycosides. The rate-determining step of the dissociation might be a conformational change of the enzyme, and it is suggested that the reaction order is the following: dissociation of the sugar portion of the cardiac glycoside from the sugar site of enzyme, conformational change of the sugar site, and dissociation of the steroid portion from the steroid site.

INTRODUCTION

The cardioactive steroids exert a specific and powerful cardiotonic action on heart muscle and have been successfully used in the treatment of heart failure. These compounds are also specific inhibitors of sodium and potassium transport and the membrane-bound ($Na^+ + K^+$)-dependent adenosine triphosphatase (EC 3.6.1.3), which is believed to be an integral part of this transport

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