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The Lack of Correlation between Hallucinogenesis and Inhibition of Axoplasmic Transport

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SUMMARY

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Several hallucinogenic indoles were examined for an inhibitory effect upon fast axoplasmic transport of proteins in the optic system of the anesthetized rat. No inhibition was produced by milligram levels of lysergide or *N*,*N*-dimethyltryptamine, although some derivatives of *N*,*N*-dimethyltryptamine produced partial inhibition at this dose. Harmaline and 6-methoxylharmalan, which are poor hallucinogens, are active as inhibitors of transport. The hallucinogenic compound *R*(−)-1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane and its nonhallucinogenic enantiomer were also tested for antitransport activity in a system *in vitro* which utilized the rat sciatic nerve. Both enantiomers are effective inhibitors of axoplasmic transport. It is concluded that inhibition of axoplasmic transport is not correlated with hallucinogenesis, even though a good correlation of these parameters has previously been observed in studies of a series of structurally related derivatives of mescaline.

INTRODUCTION

Mescaline and several of its structural derivatives have recently been described as inhibitors of rapid axoplasmic transport through the rat optic nerve (1). Within the series of drugs related to mescaline, a correlation was observed between hallucinogenic potency and antitransport activity. It is the purpose of the present investigation to test the hypothesis that inhibition of axoplasmic transport is a general property of all hallucinogens. Two experimental approaches have been used. In the first, hallucinogenic compounds which are not structurally related to mescaline were tested for antitransport activity. In the second, the recently resolved enantiomers of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane were employed as probes of antitransport activity. *R*(−)-DOM[2] exhibits twice the hallucinogenic potency of the racemic mixture, while the *S*(+) isomer has no activity as a hallucinogen (2, 3). If the correlation between antitransport activity and hallucinogenic activity were to extend to these two enantiomers, *R*(−)-DOM should be a potent

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[2] The abbreviation used is: DOM, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane.

inhibitor of transport, while its enantiomer would be inactive. Results from both experimental approaches suggest that the previously observed correlation between hallucinogenic potency and inhibition of axoplasmic transport is coincidental. In the course of this study, several new inhibitors of transport have been discovered.

METHODS

Two procedures were used for evaluating inhibitors of axoplasmic transport: an assay *in vivo* utilizing the rat optic system, and a preparation of the rat sciatic nerve *in vitro*. These preparations are described in turn.

The procedure for rapid, quantitative assessment of the effects of drugs on fast axoplasmic transport in the rat optic system has been described (1). A solution containing both tritiated L-proline and a drug of interest was injected into the vitreous humor of one eyeball of a rat. A similar solution lacking the drug was injected into the other eye. Proteins labeled with tritiated proline are transported from the retina through axons of ganglion cells at a rate of 8.4 mm/hr to the nerve terminals in the brain.[3] Three hours after the injections the level of radioactivity transported into the optic nerves is directly proportional to the amount of [^{3}H]L-proline incorporated into that fraction of the retina which is insoluble in 18% trichloracetic acid. Inhibition of axoplasmic transport could be detected by comparing the amount of radioactivity transported into the optic nerve projecting from the eye treated with the drug with the radioactivity in the nerve projecting from the control eye (containing no drug). The effect could be quantitated utilizing a correction for the amount of [^{3}H]L-proline incorporated into retinal protein, and a net transport value (NT) calculated for each experiment. A value of 1.00 indicates no inhibition of transport, while a value of 0.00 would indicate maximal inhibition. Partial inhibition would be indicated by values lying between these two extremes.

Experiments carried out in the rat optic system involved exposure of the nerve cell body to the drug being studied. As nerve cell bodies carry out many metabolic functions not found in the axon, including the large-scale synthesis of proteins, we wished to test the effects produced by a drug placed in contact only with the axons of a nerve. The system we chose to use for these experiments was a preparation of rat sciatic nerve *in vitro*, modified from that developed by Edström and Mattsson for the frog sciatic nerve (4). A sciatic nerve with the sixth and seventh lumbar dorsal root ganglia attached was dissected from a 225-g female rat anesthetized with an intraperitoneal injection of sodium pentobarbital (24 mg/kg) and urethane (600 mg/kg). The nerves were laid across the bottom of an elongated rectangular compartment separated by slotted partitions into three cells. After isolation of the cells from each other by means of silicone grease seals built up around the nerve at the partitions, the nerves were covered with tissue culture medium 199 and kept at 37° in an atmosphere of 95% O_2–5% CO_2. A typical compartment is shown in Fig. 1.

To observe axoplasmic transport, [^{3}H]L-proline was introduced into the first chamber, where it was incorporated into proteins in the two ganglia. Tritiated proteins are transported down the nerve, through the two grease seals, and accumulate at a ligation placed on the nerve in the third chamber. During the incubation, the second chamber was continually perfused with fresh medium to flush out any free tritiated proline that leaked past the grease seal. After 16–18 hr the nerve was removed and fixed with 5% trichloracetic acid (4). The nerve, which was 50% of its original length as a result of trichloracetic acid treatment, was then cut into 1-mm sections. These were solubilized with Soluene 100 and assayed for radioactivity in a Beckman LS-230 liquid scintillation spectrometer, using a scintillation mixture of 0.30% 2,5-diphenyloxazole and 0.61% benzoic acid in toluene. Scintillation counting data were corrected to disintegrations per minute by external standardized, using a Digital Equipment Corporation PDP-8/e laboratory minicomputer. Actual efficiencies of counting were 25–35%.

A discussion of the use of this experimental

[3] D. J. Schlichter and W. O. McClure, manuscript in preparation.

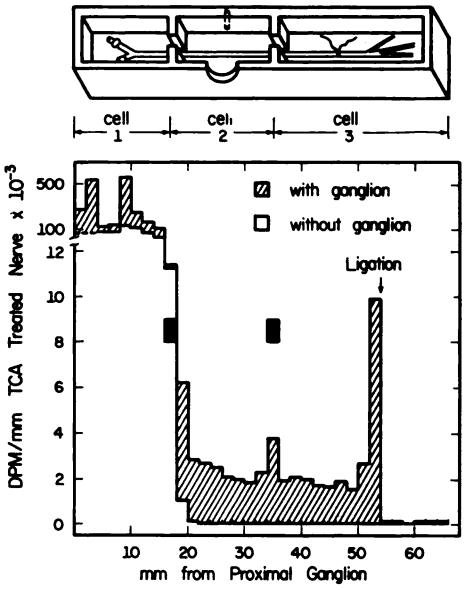

FIG. 1. *Axoplasmic transport in an apparatus in vitro*

Rat sciatic nerves were prepared and incubated as described in METHODS in a compartment (upper) containing three cells. Transport was directed from cell 1, which contained both the ganglia and [^{3}H]L-proline, toward cell 3, in which a ligation was placed in the nerve. Two experiments are shown: hatched bars, a control experiment demonstrating transport; open bars, a control experiment demonstrating the lack of transport when the ganglia were removed prior to incubation. The position of the grease seals at the two partitions is indicated by the solid blocks. Incubations were carried out for 16–18 hr at 37° and pH 7.4 in medium 199. The abscissa indicates distance from the nearer of the two ganglia, expressed in length of unfixed nerve; the ordinate represents disintegrations per minute of fixed nerve. Nerves fixed in trichloracetic acid (TCA) were one-half the length of fresh tissue. For other details, see METHODS.

system as an assay for inhibition of axoplasmic transport is given under RESULTS AND DISCUSSION and ref. 5.

Female rats of the NLR strain were procured from National Laboratories, Inc. Sodium pentobarbital was purchased from Diamond 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 μCi/μl. Soluene 100 was purchased from Packard Instrument Company. 6-Methoxyharmalan and 5-methoxy-*N*,*N*-dimethyltryptamine were purchased from Regis Chemical Company; harmaline and *N*,*N*-dimethyltryptamine, from Sigma Chemical Company; and 5-hydroxy-*N*,*N*-dimethyltryptamine, from Aldrich Chemical Company. Tissue culture medium 199 was obtained through Difco Laboratories.

RESULTS AND DISCUSSION

Inhibition of axoplasmic transport by mescaline and structurally related hallucinogens revealed a positive correlation between inhibition of transport and hallucinogenic potency (1). To see whether this correlation could be extended to all hallucinogens, including those not structurally related to mescaline, several hallucinogenic agents with structures based on indole and β-carboline were tested for inhibition of axoplasmic transport. Results obtained in the rat optic system with lysergide, *N*,*N*-dimethyltryptamine, 5-hydroxy-*N*,*N*-dimethyltryptamine, 5-methoxy-*N*,*N*-dimethyltryptamine, harmaline, and 6-methoxyharmalan are summarized in Table 1. For comparison, results obtained with mescaline are also included in Table 1. Among these drugs, no correlation between hallucinogenic potency and antitransport activity is seen. For example, lysergide, the most potent hallucinogen known, produced no inhibition of transport at levels equal to that of a full human hallucinogenic dose. *N*,*N*-Dimethyltryptamine, a drug with a hallucinogenic potency 30 times that of mescaline, showed no inhibition of transport at a dose (1.0 mg) greater than that required to produce nearly complete inhibition by mescaline.

Several of the drugs tested exhibited antitransport activity. Both the 5-hydroxy and 5-methoxy derivatives of *N*,*N*-dimethyltryptamine were active inhibitors of transport, although no more so than mescaline. Two β-carbolines were also tested. Both harmaline and 6-methoxyharmalan were effective inhibitors.

Since the correlation between antitransport activity and hallucinogenic potency reported previously (1) was based on results from a series of derivatives of mescaline, studies carried out with compounds other

TABLE 1

Effect of indole-based hallucinogens upon axoplasmic transport from rat eye

Details of the measurements are presented in METHODS.

Agent	Potency	Dose	Net transport
	mescaline units	*mg*	
Controls (N = 14)			1.01 ±0.06
Lysergide	4000	0.160	0.97
		0.080	0.72
		0.030	0.96
		0.005	1.28
		0.0009	0.75
		0.0005	0.95
Mean ± SD			0.94 ±0.2
N,*N*-Dimethyltryptamine	~30	1.0	0.96
5-Hydroxy-*N*,*N*-dimethyltryptamine		1.04	0.18
		0.50	0.44
		0.25	0.57
		0.10	0.90
5-Methoxy-*N*,*N*-dimethyltryptamine	~50	0.72	0.51
		0.63	0.73
		0.26	0.76
		0.23	0.59
		0.05	1.03
Harmaline	~1	0.15	0.09
		0.10	0.57
		0.01	0.98
6-Methoxyharmalan		0.50	0.36
		0.20	0.77
Mescaline[a]	1.0	0.40	0.50

[a] Data taken from the dose-response curve evaluated by Paulson and McClure (1).

than the methoxylated phenylalkylamines might not represent an appropriate comparison. We therefore sought to examine further the validity of the correlation within the family of methoxylated phenylalkylamines. One of these drugs, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane, the potent hallucinogen DOM, has recently been resolved into its two optically pure enantiomers (2, 3). *R*(−)-DOM is about twice as potent as the racemic mixture, while *S*(+)-DOM exhibits no hallucinogenic activity (2, 3). As the racemic mixture of DOM was found previously to inhibit axoplaxmic transport in the rat optic system, separation of the antitransport activities of the two enantiomers would further strengthen the correlation between hallucinogenic potency and antitransport activity among hallucinogens of the mescaline family.

Using the rat optic system, *R*(−)-DOM was found to be as effective an inhibitor as the racemic mixture. However, it was also found to be a good inhibitor of the incorporation of [^{3}H]L-proline into retinal protein. When 0.40 mg of *R*(−)-DOM was administered 1 hr before [^{3}H]L-proline was injected, incorporation was reduced by approximately 90%. Under identical conditions the same dose of *S*(+)-DOM had no effect on incorporation. To avoid errors in the interpretation of transport experiments in the optic system as a result of differential effects of the two isomers on incorporation of proline into retinal protein, a preparation of the rat sciatic nerve *in vitro* was employed. As described in METHODS, the sciatic nerve, with the L_6 and L_7 dorsal root ganglia attached, was placed in a chamber divided by slotted partitions into three cells (Fig. 2). After the three cells had been isolated from one another by means of grease seals built up around the nerve at the partitions, [^{3}H]L-proline could be added to the first cell, which contained the ganglia. Radioactive proteins synthesized by the ganglia were subsequently transported down the nerve. After 16–18 hr the nerve was sectioned and assayed for radioactivity.

Transport profiles of two nerves are shown in Fig. 1. One nerve, indicated by the shaded bars, depicts the levels of transport usually observed in our laboratory. Radioactive material is transported through both grease seals, shown by the solid blocks, and accumulates at a ligation (vertical arrow) previously placed on the nerve at some point in the third cell. Distal to the ligation the radioactivity drops to background levels. The slight peak

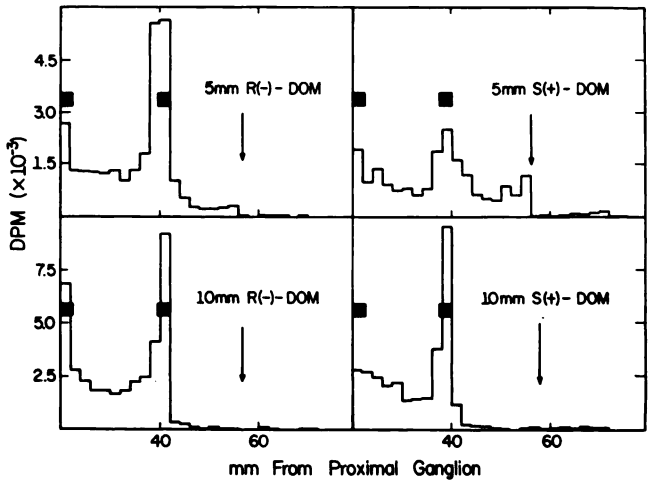

FIG. 2. *Effect of R(−)- and S(+)-DOM on fast axoplasmic transport, using sciatic nerve system in vitro of Fig. 1*

Nerves were incubated as given in METHODS with the medium in cell 3 (see Fig. 1) containing drugs at the stated concentrations. The duration of incubation was 16–18 hr. For further details, see the text and the legend to Fig. 1.

of radioactivity sometimes seen at the second solid block is due to the presence of the grease seal. In 14 such control experiments the peak of radioactivity at the second grease seal was never larger, with respect to the peak at the ligation, than that depicted in Fig. 1. A nerve from which the ganglia were removed is shown by the lower, unshaded profile. In this case no transported radioactivity was observed.

Compounds considered to be inhibitors of transport were tested in this system as follows. The drug to be studied was dissolved in tissue culture medium and placed in the third cell. The experiment was then carried out as described in METHODS. If the drug completely inhibited axoplasmic transport, the transported radioactivity could not pass through the second grease seal to accumulate at the ligation. No peak of radioactivity should be observed at the ligation, and a prominent accumulation should be present at the second grease seal. Intermediate levels of inhibition may be recognized by varying degrees of accumulation at the second seal, with a corresponding decrease in the amount of radioactive material which accumulates at the ligation. The system has previously been employed successfully to demonstrate the effect of drugs upon transport (5, 6).

Transport profiles of nerves bathed in the third cell by 5 mM and 10 mM *R*(−)- and *S*(+)-DOM are shown in Fig. 2. Both isomers inhibited transport completely as a concentration of 10 mM. Concentrations of 5 mM gave only partial inhibition. While *R*(−)-DOM produced more complete inhibition at 5 mM than did *S*(+)-DOM, the fact that both isomers were completely inhibitory at 10 mM indicates that the antitransport activities of these two drugs are separated by no more than a factor of 2.

The correlation between inhibition of axoplasmic transport and hallucinogenic potency which was observed previously with a series of mescaline derivatives is not supported by the data presented in this paper. Hallucinogens based upon the indole nucleus were examined as inhibitors of axoplasmic transport in the rat optic system. No correlation was observed between hallucinogenic potency and antitransport activity with these compounds. In addition, the resolved hallucinogenic and nonhallucinogenic isomers of DOM, a derivative of mescaline,

were studied in a system *in vitro* utilizing the rat sciatic nerve. Both compounds inhibited transport. We conclude that inhibition of axoplasmic transport is not a property of all hallucinogens, and that the correlation previously observed (1) was fortuitous.

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