Communications in Psychopharmacology, Vol.2, pp.1-10
(1978)

BRAIN AND RETINA UPTAKE OF A RADIO-IODINE LABELED PSYCHOTOMIMETIC IN DOG AND MONKEY

Thornton Sargent III, Gisela Braun, Ulrich Braun,
Thomas F. Budinger and Alexander T. Shulgin
Donner Laboratory and Lawrence Berkeley Laboratory
University of California, Berkeley 94720

Abstract

4-Iodo-2,5-dimethoxyphenylisopropylamine (4-I-DPIA) is a close analog of methoxylated psychotomimetic compounds related to 6-hydroxydopamine. Such compounds are of interest as possible endogenous psychotoxins in schizophrenia, and when labeled with a suitable gamma-emitting radioisotope their in vivo pharmacokinetics may be studied in animals or humans. 4-I-DPIA was prepared with ^{131}I and with ^{123}I and its distribution studied in dog and monkey using imaging devices of nuclear medicine. Images were obtained showing isotope accumulation in the brain and eyes in both animals; the measured uptake half-time in the monkey brain was 8 sec. Sacrifice and dissection of the dogs showed 2% of the activity in the brain, 18% in liver and 12% in lung. The concentration in retina was 5 times that in any other CNS tissue, supporting the concept that direct action at the retinal level is a component of visual misperception caused by psychotomimetics.

Introduction

The methoxylated psychotomimetic compounds such as 2,4,5-trimethoxyphenylisopropylamine (TMA-2 fig. 1c) which are structurally related to dopamine (DA, fig. 1a) and 6-hydroxydopamine (6-OHDA) (fig. 1d) are of interest because of the possible relationship between metabolic defects of catecholamine metabolism and schizophrenia. The structure activity relationships of psychotomimetic compounds structurally similar to the catecholamines have been reviewed and several theoretical mechanisms for their mode of action proposed (1). Of particular relevance to the work reported here was the observation that replacement of the 4-methoxy group with an alkyl or a halo group yielded compounds of increased potency. Replacement with a bromine atom yielded 4-bromo-2,5-dimethoxyphenylisopropylamine, 4-Br-DPIA (fig. 1e), with a potency 400 times that of mescaline (2).

The synthesis of 4-Br-DPIA provided for the first time a psychodysleptic compound which contained an atom for which there was a gamma-emitting radioisotope of suitable half-life for nuclear medicine studies. Human studies of the in vivo organ distribution of a psychodysleptic compound were performed with 4-Br-DPIA synthesized with ^{82}Br (half-life 35 hrs) (3). Two percent of the injected ^{82}Br appeared in the brain while 13 to 32% appeared in the liver and 12 to 18% in the lungs. Useful pharmacodynamic information about this psychodysleptic compound was obtained by this method, although the available bromine isotopes

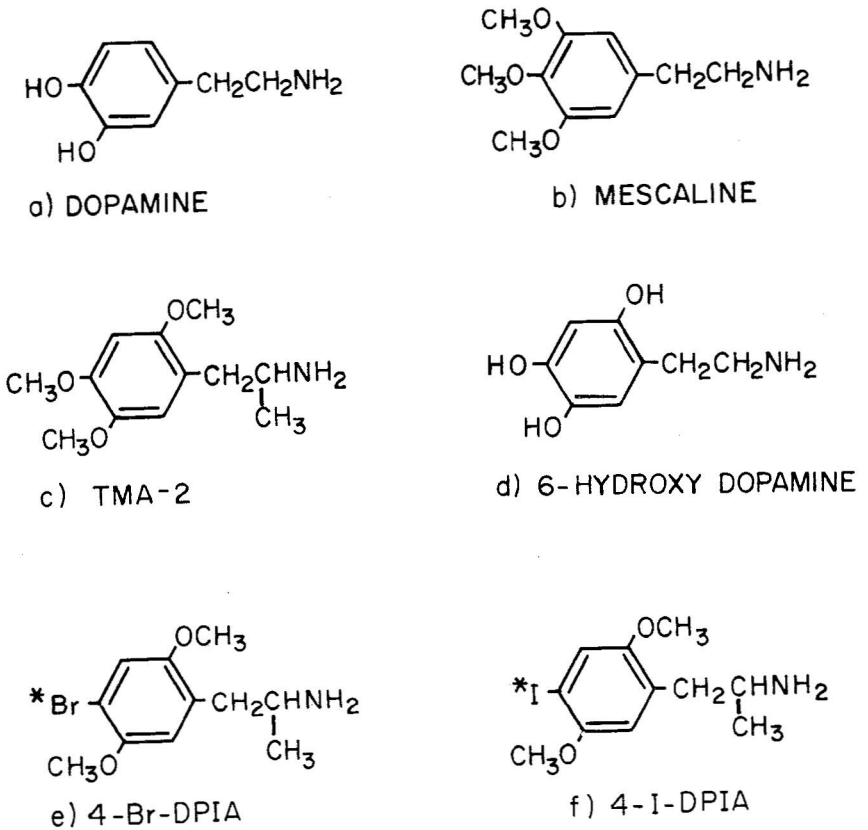

Figure 1. Structural formulae of compounds discussed.

emit high energy gamma rays that are difficult to collimate and thus the image quality and resolution are relatively poor. Further, the half-lives of these isotopes are short, and the radiation dose per useful gamma ray is relatively high. The iodine analog of a 4-Br-DPIA, 4-iodo-2,5-dimethoxyphenylisopropylamine, 4-I-DPIA (fig. 1f), has a psychotomimetic potency comparable to that of 4-Br-DPIA, (4) so we undertook the synthesis of 4-I-DPIA with radioiodine.

For our purposes the most suitable isotopes of iodine were ^{131}I 8.0 days) and ^{123}I (13.0 hrs). ^{131}I has a convenient half-life for chemical synthesis and biological studies, and it has a principal gamma ray of 364 keV which is suitable for imaging with the Anger scintillation camera. ^{123}I emits a gamma ray of 159 keV which produces better images with the scintillation camera than ^{131}I, with very little accompanying beta radia-

tion , and 50 times as many useful gamma rays as ^{131}I for the same radiation dose. Thus, the ^{123}I labeled compound is more suitable for use in human patients.

We have synthesized 4-I-DPIA with ^{131}I and with ^{123}I for studies of the organ distribution in the dog and the monkey using gamma-ray imaging methods of nuclear medicine. The structural similarity between 4-I-DPIA and DA suggests that 4-I-DPIA may exert its effect through interraction with the DA receptor. Pimozide, a recently developed neuroleptic, is believed to block dopamine receptor sites specifically (5). We therefore performed parallel studies on two dogs, one of which had been pretreated with pimozide, to determine whether the distribution of 4-I-DPIA was altered. We present the results of our experiments as an example of a new method of obtaining pharmacodynamic data by non-invasive means in the living animal.

Methods

Gamma-ray imaging was performed using two instruments developed at this laboratory: an Anger scintillation camera, a standard instrument in nuclear medicine (6), which images gamma rays of 400 KeV and less in a ten inch field of view with a spatial resolution of 9mm; and the Anger Mark II whole-body scanner (Anger. 1972), which is capable of imaging gamma rays with energies as high as 2.0 meV over the entire human body in a single scan, with a resolution of 20 mm. Data from both of these instruments are digitized and stored by an HP 5407A Scintigraphic Data Analyzer (8) and may be manipulated to yield the amount of radioactivity as a function of time by outlining any desired area of interest on the oscilloscope screen with a light pen.

The [^{131}I]-4-I-DPIA was prepared by converting Na ^{131}I to ^{131}ICl by equilibration with Icl which was then reacted with 2,5-dimethoxyphenylisopropylphthalimide (9). The iodine substitutes specifically at the four position; the phthalide group is then removed with hydrazine and the 4-I-DPIA appropriately purified for injection. The specific activity was 5.9 mCi/mM. Using ^{123}I instead of ^{131}I, a fast method of preparation of [^{131}I]-4-I-DPIA has also been described which reduced the synthesis time to 4 hrs. (10). The specific activity obtained was 35.7 mCi/mM.

Three female beagle dogs weighing 12.5 kg each were injected intravenously with [^{131}I]-4-I-DPIA. Dog 1 was scanned periodically for three days. Dog 2 served as control for dog 3 which was given an oral dose of 40 mg of pimozide 3-1/2 hrs prior to injection. Each dog received 0.67 mCi of ^{131}I containing approximately 40 mg of 4-I-DPIA brought to isotonicity with saline and sterilized by filtration. The dogs were anesthetized with Diabutal to maintain immobility during scanning. Within 3 min after injection of 4-I-DPIA spastic abdominal breathing occurred, and the forelegs became rigid; these effects had disappeared by 20 min. Gamma-ray scintigraphy was performed with the whole-body scanner and the scintillation camera, and the data stored in the HP5407A.

Three hrs after injection dogs 2 and 3 were sacrificed with euthanol. The dissected organs and remaining carcasses were scanned on the whole-body scanner to obtain quantitative uptake data. Regions of interest in the scans were outlined by light pen and the total counts in the

region were calculated by the HP5407A. The brain was removed from the calvarium and sliced into approximately 1 cm transverse sections which were imaged with the scintillation camera. Small organs and samples of brain tissue were placed in tared vials and counted in a well scintillation counter; values from the well counter and the scanner were compared to the injected dose. A male bonnet monkey (Macaca radiata, 7.5 kg) was anesthetized with pentobarbital, placed in position under the scintillation camera, and injected i.v. with 3.5 mCi [^{123}I]-4-I-DPIA. Data were accumulated and subsequently framed into two second intervals for evaluating the changes in brain activity as a function of time.

Results

Scintillation camera images of dog 1 are presented in fig. 2; radioactivity is seen in the brain in lateral views at 2½ and 6 hr, with a smaller amount in the area of the thyroid. In the 6 hr vertex view, and in the 72 hr lateral view, radioactivity can be seen clearly in the eyes. At 72 hr there is also activity in the thyroids and in the parotid glands, but the activity in the brain has disappeared. Scintillation camera pictures of the brain slices did not indicate any remarkable differential uptake of 4-I-DPIA between cortex and mid-brain.

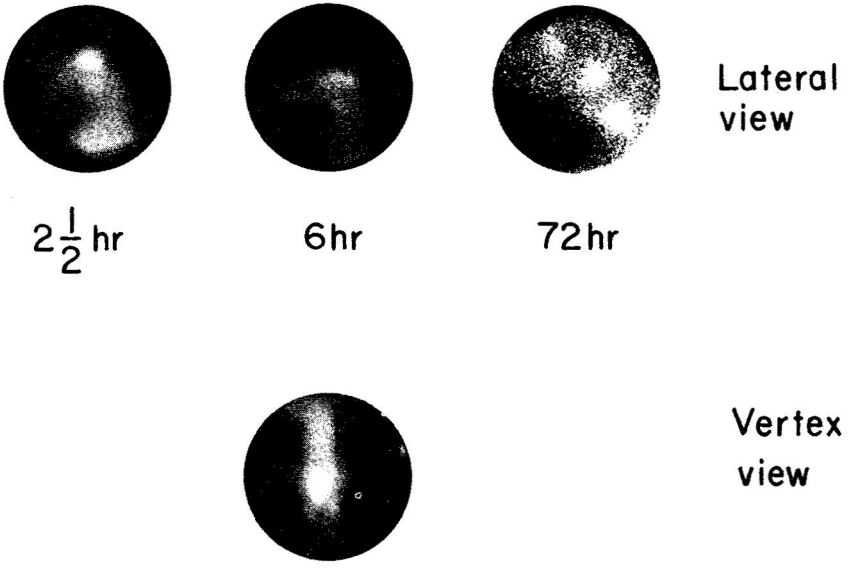

Figure 2. Scintillation camera images of dog 1 at various times after administration of [^{131}I]-4-I-DPIA.

TABLE I

Content of ^{131}I in dissected organs of dose 1 and 2, as percent of total dose in each organ, percent of total dose per gram of organ weight, and the percent change from the control dog 2 to the Pimozide treated dog 3.

		Dog 2, Control		Dog 3 Pimozide		% Change	
		% dose organ	% dose gm	% dose organ	% dose gm	% dose organ	% dose gm
Scanner	Carcass	52.9		55.7		+6	
	Gut	10.5		8.2		-23	
	Lungs	11.8	0.134	8.9	0.100	-25	-25
	Liver	18.7	0.048	21.9	0.056	+17	+17
	Heart	1.1	0.012	.9	0.010	+18	+18
	Spleen	1.0	0.035	.7	0.024	-30	-31
	Kidneys	1.2	0.021	1.0	0.018	-20	-14
	Brain	2.0	0.020	2.1	0.021	+5	+5
	Eyes	0.2	0.014	0.25	0.019	+25	+36
Well counter	Retina (One)	0.026	0.098	0.038	0.113	+46	+15
	Lacrimal glands	0.0026	0.0013	0.0056	0.0025	+115	+92
	Adrenals	0.014	0.0052	0.0102	0.0040	-27	-23
	Ovaries	0.0051	0.0018	0.0066	0.0016	+29	-11
	Thyroids	0.0099	0.012	0.019	0.026	+90	+91
	Pituitary	0.0005	0.011	0.0004	0.011	-20	+6
	Caudate nucleus		.0102		.0092		+10
	Cerebellum white matter		.0080		.0075		-6
	Cerebellum grey matter		.0109	.0082			-25
	Hypothalamus		.0089		.0078		-12
	Olfactory and frontal		.0103		.0115		-8
	Cerebral cortex, white		.0118		.0109		+12

The data from the regions of interest obtained by the computer from the scans of the dissected organs of dogs 2 and 3 are shown in Table 1. Also shown in Table are the activities found in small organs and tissue samples by well scintillation counting. The major fraction of the radioactivity at the time of sacrifice was in the liver followed by the lung. The brain contained 2% while the eyes, where uptake was prominent in scans at later times in dog 3, contained 0.2% of the injected dose. The most striking tissue concentration, however, was in the retina; it was exceeded only by that of the lung, and was five times greater than the concentration in whole brain. Eye tissues other than retina, and the lacrimal glands, did not concentrate activity to any significant extent. The brain tissues sampled showed uniform concentrations of activity, which was one-tenth of that in the retina. The very small amount of activity in the thyroids, 0.01-0.02%, indicates that no more than 0.07% of the 4-I-DPIA molecule was cleaved to ionic iodide, assuming an iodide uptake by the gland of 30%.

The isolated retina was subjected to an extraction procedure designed to provide an estimate of the chemical form of the ^{131}I found there. The extraction and the results are outlined in Figure 3. Seventeen percent of the activity was solubilized by homogenization and was not precipitated by either $HClO_4$ or $AgNO_3$, probably representing unchanged

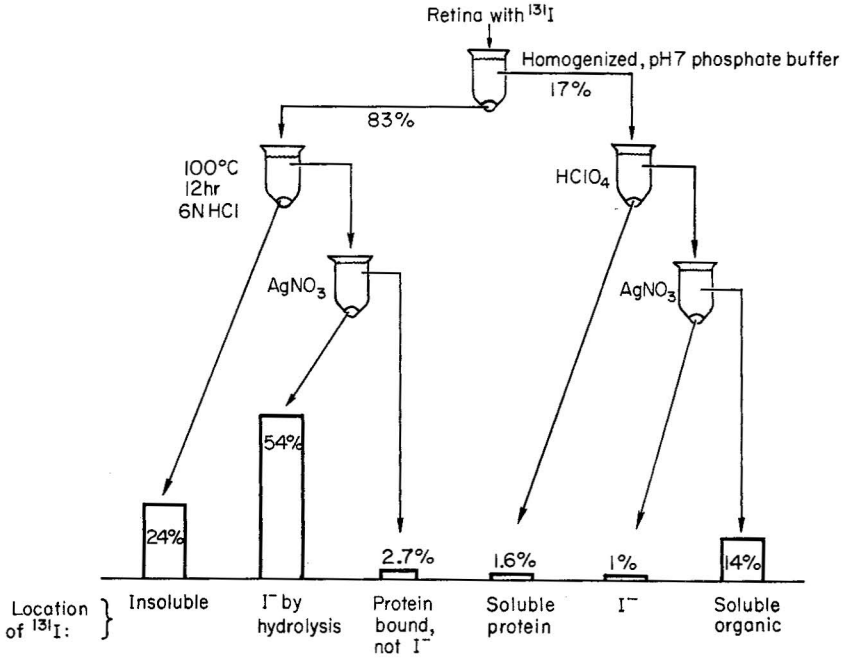

Figure 3. Outline of separation and extraction scheme for retina, showing percent of total radioactivity in each fraction.

4-I-DPIA and its metabolites. The remaining 83% of the activity in the pellet was found to be largely solubilized by acid hydrolysis, and was released as inorganic iodide as shown by Ag^{+} precipitation.

The uptake of the ^{123}I labeled 4-I-DPIA in the brain of the monkey is shown in Fig. 4. Scintillation camera pictures during the early phase are shown related to specific times on the curve. The ^{123}I is clearly taken up by brain, and the uptake half-time was 8 seconds. After 24 hr activity is seen in the eyes and in the thyroid, but the parotid glands are not apparent as they were in the dog; the brain activity has diminished compared to the eye activity at this time.

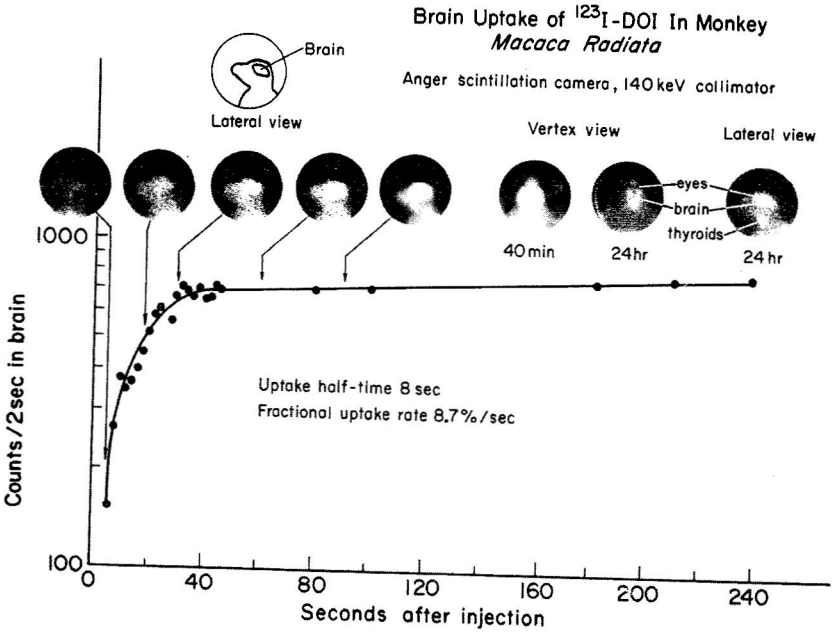

Figure 4. Uptake of radioactivity in brain of monkey after injection of [^{123}I]-4-I-DPIA. Counts in successive 2 sec intervals within the outlined area indicated are plotted as a function of time after injection. Scintiphotos are keyed to the time at which they were taken.

Discussion

4-I-DPIA is selectively taken up by brain in the dog and monkey in a fashion comparable to that seen in humans with its analog 4-Br-DPIA. The extraction from blood to brain in the monkey has a half-time of eight seconds, which is the same as the blood-brain perfusion time (11). With such short uptake time it seems unlikely that there could be extensive metabolic changes exerted on the molecule, and hence, the radioactivity seen initially in the brain probably represents unchanged 4-I-DPIA. The unusual uptake in the retina was not seen until somewhat later, although an earlier appearance of activity could have been obscured in the image by the higher brain uptake.

In the retinal extraction procedure (fig. 3.) the soluble fraction contained 17% of the radioactivity. This may represent unchanged 4-I-DPIA or water soluble metabolites, but not de-iodinated products as the amount of free iodide present was only 1%. An effort was made to free the 4-I-DPIA or its metabolites from the particulate fraction which remained after homogenization. Vigorous hydrolysis succeeded in solubilizing 54% of the ^{131}I as iodide, but 24% still remained bound. Rotman et al. (12) have shown a strong binding of 6-OHDA or its metabolites to a number of protein substrates. Jacob and Castagnoli (13) have shown a similar binding of the hydroquinone metabolite of DOM, the 4-methyl analog of 4-I-DPIA. It seems likely that 4-I-DPIA undergoes a metabolic conversion similar to that known for DOM, namely bis-O-demethylation and conversion to the hydroquinone without affecting the 4-substituent. If so, the same mechanism of protein binding could also occur for 4-I-DPIA in the retina, with the product retaining the radioactive iodine when bound.

This finding of uptake of radioactivity in the retina poses the possibility previously suggested by others that perceptual distortions produced by psychotomimetic compounds might occur at the level of the sensing organ rather than at, or in addition to, higher cortical associative levels. For example, Shah et al. (15) have reported a high concentration of [^{14}C]-mescaline in the eye of the mouse. Further studies to elucidate the exact cellular location might be best conducted using [^{125}I]-4-I-DPIA and autoradiography of retina. The structural similarity between DA and 4-I-DPIA is of interest here because DA neurons have been found in the retina (16), although their function is not known. Activity was also seen in the thyroid and parotid glands of the dog at later times, and although iodide is known to be taken up by these glands, it does not accumulate in the retina.

The distribution of 4-I-DPIA in the organs of the dog showed that the largest uptake was in the liver, a not unexpected result in view of the function of this organ in metabolic degradation of foreign materials. Since a comparable amount was found in the gut, it is possible that the iodine-containing metabolites are excreted via the bile. The high total uptake and high concentration of 4-I-DPIA in the lung is probably related to the fact that the lung is a prominent site of amine metabolism

(17). The 2% uptake in the brain was greater than that in heart or adrenal glands, both sites of catecholamine activity.

Comparison of the control dog and the pimozide treated animal shows no significant change in brain uptake. Increased uptake relative to the control dog was seen in the liver, heart, eyes, retina, lacrimal glands, and thyroid with decreased uptake in most other organs. It appears that with the blocking of uptake of 4-I-DPIA in catecholamine rich organs (lungs, adrenals) by pimozide, there is a consequent shunting of 4-I-DPIA to degradative metabolism. This is suggested by the increased uptake after pimozide in organs involved in metabolic degradation, i.e., liver, and the increased uptake of released iodide by the thyroid. The lack of differential uptake in various brain areas, coupled with the lack of effect of pimozide on 4-I-DPIA distribution in brain, suggests that if the action of 4-I-DPIA is related to dopamine uptake mechanisms, this effect is not manifested by its organ distribution. Presulable the psychodysleptic properties of such compounds involve action at specific points at the cellular level in the central nervous system, but this may occur in the presence of a relatively uniform distribution of the compound.

The methods of nuclear medicine as demonstrated here are capable of providing in vivo measurements which, when coupled with more detailed knowledge of the biochemical changes and dispensation of the radionuclide label, should provide a useful method for investigating the etiology of mental disease; they are directly applicable to study of human subjects.

Acknowledgment

Natalia Kusubov, Brian Moyer and Sandra Stoddard rendered valuable technical support. Dr. Ulrich Braun was supported by the Deutsche Forschungsgemeinschaft. This work was supported by the U.S. Department of Energy (DOE).

References

1. Shulgin, A.T., Sargent, T., and Naranjo, C., Nature, 221, 537-541 (1969).

2. Shulgin, A.T., Sargent, T., and Naranjo, C., Pharmacology 5, 103-107 (1971).

3. Sargent, T., Kalbhen, D.A., Shulgin, A.T., Braun, G., Stauffer, H., and Kusubov, N., Neuropharmacol. 14, 165-174 (1975).

4. Shulgin, A.T., in Handbook of Psychopharmacology, section III, Human Psychopharmacology, edited by Iversen, Iversen and Snyder, New York: Plenum Press, (in press).

5. Soudijn, W., and Van Wijngaarden, I., J. Pharm. Pharmac. 24, 773-780 (1972).

6. Anger, H.O., Instrumentation in Nuclear Medicine, edited by Gerald J. Hine, pp. 485-552. New York: Academic Press (1967).

7. Anger, H.O., Hospital Practice, 7, 45-54 (1972).

8. Budinger, T.F., Symposium on Medical Radioisotope Scintigraphy, Monte Carlo, Vol. 1, IAEA, Vienna, (1972) pp. 501-555.

9. Braun, U., Shulgin, A.T., Braun, G., and Sargent III, T., J. Med. Chem., 20, 1543-1546, 1977.

10. Braun, G., Shulgin, A.T., and Sargent III, T., J. Labelled Cmpd. Radiopharm. (in press).

11. Kety, S.S., and Schmidt, C.F., J. Clin. Invest., 27, 476-484 (1948).

12. Rotman, A., Daly, J.W., and Creveling, C.R., Mol. Pharmacol. 12, 887-899 (1976).

13. Jacob, P. and Castagnoli, N., University of California, San Francisco, unpublished data.

14. Brawley, P., and Duffield, J.C., Pharmacol, Rev., 24, 31-66 (1972).

15. Shah, N.S., Neely, A.E., Shah, K.R., and Lawrence, R.S., J. Pharm. Exper. Therap., 184, 489-493 (1973).

16. Laties, A.M., Investigative Opthalmology

17. Fowler, J.S., Gallagher, B.M., MacGregor, R.R., and Wolf, A.P., J. Pharm. Exp. Therap., 198, 133-139 (1976).