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EVIDENCE THAT DEPRENYL, A TYPE B MONOAMINE OXIDASE INHIBITOR, IS AN INDIRECTLY ACTING SYMPATHOMIMETIC AMINE

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Abstract—Various doses of *l*-deprenyl were tested for their abilities to increase blood pressure or heart rate in animals pretreated with a ganglionic blocking agent. The use of a ganglionic blocker (chlorisondamine) ensured that deprenyl-induced responses were mediated by the peripheral autonomic nervous system, and that these responses were not influenced by the central nervous system. It was found that *l*-deprenyl had a modest ability to increase blood pressure and a marked ability to increase heart rate. The ability of *l*-deprenyl to increase heart rate was diminished or abolished by propranolol, reserpine, and chemical sympathectomy (6-hydroxydopamine). Deprenyl-induced responses were negligibly affected by adrenalectomy. These data suggest that *l*-deprenyl is an indirectly acting sympathomimetic amine whose responses are mediated by norepinephrine in postganglionic sympathetic neurons. An additional finding was that desmethylimipramine, a known blocker of the norepinephrine pump, antagonized the effects of *l*-deprenyl. This finding suggests that *l*-deprenyl enters sympathetic neurons via the membrane pump. The ability of *l*-deprenyl to enter sympathetic neurons and evoke release of endogenous amines was not accompanied by a significant loss of tissue (heart) norepinephrine.

There is both biochemical and pharmacological evidence to suggest that the enzyme monoamine oxidase (MAO) exists in multiple forms (see reviews in Refs. 1–3). Perhaps the most compelling evidence arises from the finding that there are selectively acting inhibitors of MAO. Studies with these inhibitors have led to the suggestion that MAO exists in at least two forms. For sake of simplicity, these forms (isoenzymes?) have been labeled type A and type B. In terms of selective inhibition, it has been found that clorgyline is a preferential inhibitor of the type A enzyme [4], and deprenyl is a preferential inhibitor of the type B enzyme [5].

The existence of multiple forms of MAO could mean that the individual species have a unique localization or a unique function. A number of investigators have examined this possibility by administering one or the other of the major inhibitors (i.e. clorgyline or deprenyl), and then monitoring evoked changes in the nervous system (e.g. tissue levels of catecholamines) or in behavior (e.g. spontaneous motor activity). In essence, these investigators have used MAO inhibitors as pharmacological tools. An examination of the literature reveals that this approach is being used in several laboratories (see review in Ref. 6).

The value of using MAO inhibitors as pharmacological tools is based on the premise that these drugs are specific in their mechanisms of action. The purpose of the present study is to report that, insofar as deprenyl is concerned, such specificity may be elusive. Data are presented which show that deprenyl, in addition to being a type B inhibitor, is also an indirectly acting sympathomimetic amine. This finding has implications for interpreting the effects of deprenyl on the nervous system and on behavior.

MATERIALS AND METHODS

Animals. Male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) weighing between 200 and 275 g were used in these studies. Animals were subjected to various biochemical or cardiovascular procedures that are described below.

Recording of blood pressure and heart rate. The techniques used for recording cardiovascular responses have been described in detail [7, 8]. In brief, the procedures were as follows. Blood pressure was monitored through a cannula (PE50) installed in the left carotid artery. Blood pressure recordings were obtained by using a pressure transducer (Hewlett Packard 1280B) and pressure amplifier (Hewlett Packard 8805C) connected to a thermal recording system (Hewlett Packard 7754A). Heart rate was monitored with two subdermal electrodes (active leads) in the chest wall and one subdermal electrode (reference lead) in a hind limb. Heart rate recordings were obtained by coupling a rate computer (Hewlett Packard 8812A) with a bioelectric amplifier (Hewlett Packard 8811A), both of which were connected to the recording system.

During experiments on blood pressure and heart rate, animals were anesthetized (pentobarbital sodium, 50 mg/kg, i.p.). In addition, animals were

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pretreated with a muscarinic cholinergic blocking agent (atropine, 25 mg/kg, i.p.) and with a ganglionic blocking agent (chlorisondamine, 1.0 mg/kg, i.p.). These pretreatments ensured that all tonic efferent activity and all reflex activity were eliminated. This in turn served to 'isolate' the autonomic nervous system from the influences of the central nervous system.

Adrenalectomy and sympathectomy. The adrenal glands were removed under ether anesthesia. Bilateral incisions were made through the skin and muscle wall overlying the kidneys. The adrenals were located and removed by teasing. After adrenalectomy, skin and muscle were closed with surgical staples. The operation was rapid (~ 10 min) and produced little bleeding. After the operation, animals were maintained on a normal diet plus saline (0.154 M NaCl) that contained terramycin (~ 0.5 g/l). Animals were used 1 week after adrenalectomy.

A separate group of animals was chemically sympathectomized with 6-hydroxydopamine (6-OHDA).

A dose of 50 mg/kg was given on day 1, and an additional dose of 100 mg/kg was given on day 2. Studies on evoked cardiovascular responses or on tissue levels of norepinephrine were conducted on day 4. 6-OHDA was administered in a saline vehicle (0.154 M NaCl) that contained ascorbic acid (0.01 M). The mixture was prepared immediately prior to use. Animals were injected via the tail vein.

Tissue levels of norepinephrine (NE) and uptake of radioactive norepinephrine [^{3}H]*l-NE*). Endogenous NE was assayed by the trihydroxyindole method [9] as subsequently modified by Shellenberger and Gordon [10]. Animals were sacrificed by decapitation, and hearts were rapidly removed, washed and blotted dry. After being weighed, tissues were homogenized in iced 0.5 N perchloric acid and then centrifuged (5000 rev/min, 10 min, Fisher clinical centrifuge). The supernatant was applied to an alumina column (pH adjusted to ~ 8.6), and three washes (distilled water) were performed to remove nonadsorbed substances. NE was subsequently eluted with acetic acid (0.5 N). The acid eluate was analyzed for NE spectrofluorimetrically. Data were corrected for recovery.

Uptake of radioactive NE into heart was studied after the administration (femoral vein) of tracer doses (0.15 μg/kg) of [^{3}H]*l*-NE (saline vehicle) to rats. Fifteen min after [^{3}H]*l*-NE injection, animals were decapitated. Their hearts were removed rapidly and treated identically to hearts analyzed for endogenous NE. [^{3}H]*l*-NE was assayed by liquid scintillation spectrometry. Data were corrected for recovery.

Drugs. The drugs used were: *l*-deprenyl HCl (gift from Warner-Lambert Co., Morris Plains, NJ), reserpine (gift from Smith Kline & French Laboratories, Philadelphia, PA), desmethylimipramine HCl (gift from USV Pharmaceutical Corp., Tuckahoe, NY), chlorisondamine chloride (gift from Ciba Pharmaceutical Co., Summit, NJ), [^{3}H]*l*-norepinephrine (5.85 Ci/m-mole, New England Nuclear, Boston, MA), *l*-norepinephrine bitartrate (Winthrop Laboratories, New York, NY) and *d,l*-propranolol HCl, 6-hydroxydopamine HBr and atropine (Sigma Chemical Co., St. Louis, MO). All drug dosages are expressed as the free base.

Data. Individual data points on each figure represent the mean ± standard deviation (S.D.) of at least four observations. Likewise, data referred to in the text represent the mean ± S.D. Statements on statistical analyses (P values) refer to use of the Student's *t*-test.

RESULTS

Effect of l-deprenyl on blood pressure and on heart rate. Individual groups of rats (n = 4 or more) received one of several doses of *l*-deprenyl, after which either blood pressure or heart rate responses were monitored. The doses of *l*-deprenyl that were tested ranged from 0.1 to 10.0 mg/kg (i.v.). As shown in Fig. 1, *l*-deprenyl evoked both pressor and tachycardiac responses. Figure 1 also shows that heart rate responses were much more pronounced. Accordingly, the effects of *l*-deprenyl on *β*-adrenergic transmission were examined in detail.

β-Adrenergic activity. Figure 1 indicates that the ED_{50} for deprenyl-induced responses is about 2.0 mg/kg. When this dose was administered to animals (n = 5), the average heart rate response was 87 ± 9 beats/min (bpm). Pretreatment of animals (n = 4) with *d,l*-propranolol (1.0 mg/kg, i.v., 30 min) essentially abolished this response (< 5 bpm). Furthermore, the administration of *d,l*-propranolol (1.0 mg/kg, i.v.) 15 min after *l*-deprenyl (2.0 mg/kg, i.v.) caused tachycardia to disappear.

Interaction with reserpine. The role of endogenous catecholamines in deprenyl-induced responses was assessed by using reserpine. When administered at a dose of 1.0 mg/kg (i.p., 16–20 hr), reser-

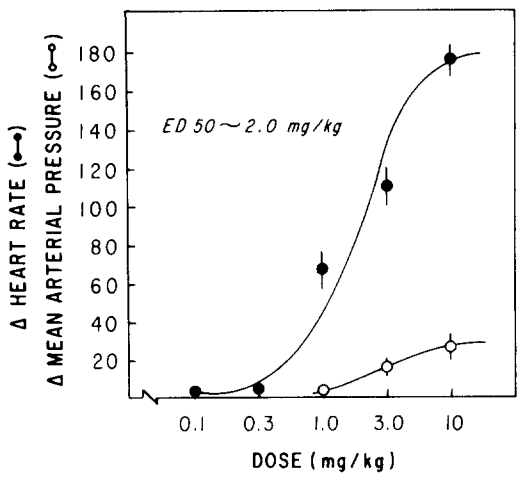

Fig. 1. Average change (Δ) in mean arterial pressure and the average change in heart rate evoked by various doses of *l*-deprenyl. Each point on the figure represents the mean response (± S.D.) obtained from four or more animals. Each animal received only one injection (i.v.) of *l*-deprenyl. Heart rate responses were much more pronounced than blood pressure responses. Nevertheless, the ED_{50} for both responses was about 2.0 mg/kg. A dose of 10 mg/kg was the maximum dose practical for testing; higher doses tended to be toxic.

pine caused marked depletion of cardiac NE. The values obtained in control and in reserpinized animals were, respectively, 806 ± 74 and 83 ± 32 ng/g. The difference is statistically significant ($P < 0.001$).

When animals (n = 4) were pretreated with reserpine (as above), responses to *l*-deprenyl (2.0 mg/kg, i.v.) were greatly attenuated. The responses in control and in reserpinized animals were, respectively, 93 ± 12 and 9.5 ± 6.7 bpm. The difference is statistically significant ($P < 0.001$).

Adrenalectomy. The striking effect of catecholamine depletion on deprenyl-induced responses could implicate either the adrenal glands or postganglionic sympathetic neurons. A putative role for the adrenals was tested by administering *l*-deprenyl (2.0 mg/kg, i.v.) to adrenalectomized animals (n = 6). The average response evoked in these animals was 71 ± 15 bpm. In matched controls, the evoked response was 86 ± 6 bpm. This difference does not attain statistical significance.

Sympathectomy. *l*-Deprenyl (2.0 mg/kg, i.v.) was administered to animals (n = 4) pretreated with 6-OHDA. In such animals, deprenyl-induced heart rate responses were 3.3 ± 4.4 bpm, i.e. essentially nil. In related experiments, tissue levels of cardiac NE were determined in control animals and in sympathectomized animals (n = 4 each). The respective values were 754 ± 126 and 181 ± 103 ng/g. The difference is statistically significant ($P < 0.001$).

Temporal aspects of l-deprenyl activity. Regardless of whether *l*-deprenyl was administered to control animals or to experimental animals (adrenalectomy, sympathectomy or drug pretreatment), evoked responses attained a peak within

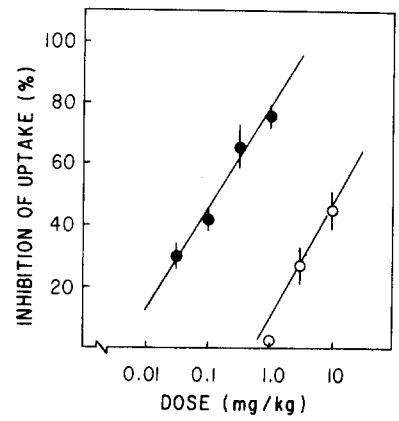

Fig. 2. Per cent inhibition of [^{3}H]*l*-NE uptake into heart. Various doses of desmethylimipramine (●—●) or of *l*-deprenyl (○—○) were administered to rats (n = 4 or more per data point), after which [^{3}H]*l*-NE was administered (i.v.). Uptake of radioactive NE was compared in experimental animals (desmethylimipramine or *l*-deprenyl pretreatment) and in control animals. Per cent inhibition of uptake was calculated from the equation: % Inhibition = $100 - [(\text{uptake}_{exp}/\text{uptake}_{con}) \times 100]$. Desmethylimipramine was more potent than *l*-deprenyl in blocking uptake of [^{3}H]*l*-HE. (Note: These data should *not* be used to make quantitative comparisons relating to drug affinity for the norepinephrine pump. The reader is reminded that the experiments were conducted *in vivo*, and thus the data are a function of both affinity for the pump and disposition of drug.)

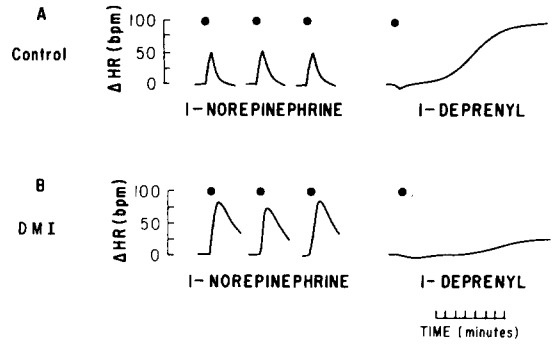

Fig. 3. Effect of desmethylimipramine (DMI) on responses to *l*-norepinephrine and to *l*-deprenyl. In panel A are illustrated the responses to a series of injections of *l*-norepinephrine (0.1 μg/kg, i.v., 10-min intervals) and a single injection of *l*-deprenyl (2.0 mg/kg, i.v.) to a control animal. In panel B are illustrated the same types of responses in an animal pretreated (30 min) with DMI (0.3 mg/kg, i.p.). Note that DMI increased the magnitude and duration of responses to *l*-norepinephrine, but it decreased the magnitude of responses to *l*-deprenyl.

10–15 min. In the present study, no attempt was made to relate response duration to blood levels of drug. However, preliminary observations indicated that half-decay times for evoked responses (e.g. 2.0 mg/kg) were greater than 2 hr.

Interaction with desmethylimipramine (DMI). Various doses of DMI (i.p., 30 min) were tested for their ability to block uptake of [^{3}H]*l*-NE into heart. The ID_{50} for DMI-induced blockade of uptake was between 0.1 and 0.3 mg/kg (Fig. 2). A dose of 0.3 mg/kg was used in subsequent studies.

Animals were prepared for recording of heart rate responses. A control group of animals received a series of injections of *l*-NE (0.1 μg/kg, i.v.), after which a single injection of *l*-deprenyl (2.0 mg/kg, i.v.) was given. An experimental group of animals was treated identically, except that DMI (0.3 mg/kg, i.p.) was given as a pretreatment (30 min). A representative experiment is illustrated in Fig. 3. In control animals, responses to *l*-NE averaged about 50 bpm, and responses to *l*-deprenyl averaged about 92 bpm. After DMI pretreatment, responses to *l*-NE were increased in magnitude (85 bpm) and in duration. By contrast, responses to *l*-deprenyl were much reduced (24 bpm). Both the increase in responsiveness to *l*-NE and the decrease in responsiveness to *l*-deprenyl were statistically significant ($P < 0.01$).

Tissue levels of NE and uptake of [^{3}H]*l-NE. l*-Deprenyl was tested at several doses (0.1 to 10.0 mg/kg, i.v., 30 min) for its ability to increase or decrease heart levels of NE. At no dose did *l*-deprenyl alter endogenous levels of NE. On the other hand, *l*-deprenyl did have an effect on uptake of exogenous NE. When administered 15 min prior to [^{3}H]*l*-NE (0.15 μg/kg, i.v.), *l*-deprenyl (i.v.) produced a dose-dependent blockade of NE uptake into heart (Fig. 2).

DISCUSSION

Drugs that affect behavior are usually examined for their effects on the peripheral autonomic ner-

vous system. Such studies are thought to be appropriate for a variety of reasons, one of which is particularly germane to this report. By studying the actions of a drug on the autonomic nervous system, one might learn something that would apply to the central nervous system (CNS). This information could lead to an understanding of the cellular events that culminate in a drug-induced behavioral response.

Unlike most psychoactive drugs, *l*-deprenyl has received only limited attention in terms of its autonomic effects. This lack of attention must be viewed in the context that *l*-deprenyl has been used as a pharmacological tool to study the role of CNS amines [6]. It has also been used to treat depressive illness [11] and to treat Parkinson's disease [12]. All of these uses are linked to the ability of *l*-deprenyl to inhibit type B MAO. However, the present study demonstrates that *l*-deprenyl is not solely an MAO inhibitor. An examination of the autonomic effects of *l*-deprenyl indicates that the drug is an indirectly acting sympathomimetic amine.

As Fig. 1 illustrates, *l*-deprenyl has a marked ability to increase heart rate, but it has only a modest ability to increase blood pressure. In light of these findings, attention has been focused on the heart rate effects of the drug. In work to be reported elsewhere (J. Bilezikian, personal communication), it has been found that *l*-deprenyl is not a β-agonist. On the other hand, deprenyl-induced tachycardia can be diminished or abolished by propranolol. This sequence of findings suggests that the effects of *l*-deprenyl are mediated by endogenous catecholamines. The fact that reserpine pretreatment nearly abolishes responses to *l*-deprenyl further implicates endogenous amines.

If *l*-deprenyl were an indirectly acting amine, i.e. an agent that evokes the release of endogenous catecholamines, then either adrenalectomy or sympathectomy should alter responses. In reality, adrenalectomy had only a negligible effect. By contrast, sympathectomy had a striking effect. Of the various procedures that were used, 6-OHDA-induced sympathectomy was the most effective in diminishing responses to *l*-deprenyl. This finding indicates that postganglionic sympathetic neurons are the source of catecholamines through which *l*-deprenyl evokes responses.

There are actually several possible mechanisms by which *l*-deprenyl might act. Three of these mechanisms are worthy of consideration. First, *l*-deprenyl might diffuse across the neuronal membrane. Once inside the nerve ending, it might evoke transmitter release. Second, *l*-deprenyl might be a substrate for the membrane pump that captures NE and related compounds and transports them into the nerve. Once again, *l*-deprenyl might act intraneuronally to evoke transmitter release. Third, *l*-deprenyl might act mainly on the external surface of the nerve membrane as an inhibitor of the NE pump. In so doing, *l*-deprenyl would inhibit the major mechanism for inactivating NE, and thus increase the amount of NE available to interact with postjunctional receptors.

A decision among these three possibilities can be made by using a known inhibitor of the NE pump, namely, DMI. If the first possibility were true, then *l*-deprenyl and DMI should act synergistically. NE released by *l*-deprenyl would be protected from inactivation by DMI. If the second possibility were true, then *l*-deprenyl should be antagonized by DMI. Blockade of the NE pump would prevent *l*-deprenyl from reaching its site of action. If the third possibility were true, then *l*-deprenyl and DMI should act additively. The two drugs would act jointly to inactivate the pump and thereby increase availability of NE.

The data illustrated in Fig. 3 leave little doubt about the most plausible conclusion. When DMI was combined with the ED_{50} for *l*-deprenyl, responses to the latter were notably antagonized. This occurred in the presence of clear evidence that DMI was blocking the NE pump (Fig. 2) and that DMI was potentiating the actions of NE (Fig. 3). It appears that *l*-deprenyl enters postganglionic sympathetic neurons via the NE pump, and that it subsequently evokes the release of neuronal catecholamines. The importance of passive diffusion across the membrane or blockade of the membrane pump must be minimal.

A question that has not been answered is that of why *l*-deprenyl exerts greater effects on sympathetic neurons in heart as opposed to those in vasculature. A tentative explanation could be the dual possibility that: (a) *l*-deprenyl has a relatively low affinity for the NE pump (see Fig. 2 and Ref. 13), and (b) sympathetic nerves in heart may have a more avid uptake mechanism than sympathetic nerves in vasculature. The matter requires further study.

Another question of note pertains to the CNS. The data in this report deal exclusively with the peripheral nervous system. It is not known whether *l*-deprenyl is an indirectly acting amine in the brain. However, as interest in *l*-deprenyl mounts, and as the frequency of its use as an investigational tool increases, studies to determine whether *l*-deprenyl is an indirectly acting amine in the brain must be undertaken. In the absence of such work, the neurological and behavioral effects of *l*-deprenyl may be difficult to explain.

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