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Pharmacological comparison between the actions of methamphetamine and 1-aminoindan stereoisomers on sympathetic nervous function in rat vas deferens

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Abstract

The selective monoamine oxidase-B inhibitor selegiline (deprenyl) causes sympathomimetic effects and is metabolised to *R*(−)-methamphetamine and *R*(−)-amphetamine. The new monoamine oxidase-B inhibitor rasagiline is devoid of sympathomimetic effects and is metabolised to *R*(+)-1-aminoindan. Sympathomimetic effects of methamphetamine and 1-aminoindan enantiomers were compared in the rat vas deferens. *R*(−)-methamphetamine and *S*(+)-methamphetamine caused initial potentiation and subsequent inhibition of the field stimulation-induced twitch response of isolated rat vas deferens (0.1 Hz). EC_{50} values for inhibition of twitch in prazosin-treated vas deferens were 0.36 ± 0.13 and 1.64 ± 0.10 μM (mean ± S.E.M.) for *S*(+)- and *R*(−)-methamphetamine, respectively. There was no difference between *S*(+)-methamphetamine and *R*(−)-methamphetamine in potentiation of postsynaptic contractile response to noradrenaline. *R*(+)- and *S*(−)-1-aminoindan increased twitch response only at concentrations above 30 μM. *R*(−)-methamphetamine has similar potency to *S*(+)-methamphetamine in potentiation of noradrenaline-mediated responses and can therefore play a role in the sympathomimetic effects of selegiline.
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1. Introduction

The amphetamine derivative selegiline (deprenyl) was developed by Knoll et al. (1965) as an antidepressant drug with psychoactivating properties, but was subsequently found to be a selective inhibitor of monoamine oxidase-B (Knoll and Magyar, 1972). Inhibition of monoamine oxidase-B reduces dopamine metabolism in human basal ganglia (Riederer et al., 1978), and selegiline exerts a dopaminergic symptomatic effect in treatment of Parkinson's disease (Palhagen et al., 1998). Selegiline possesses psychoactivating and sympathomimetic actions (Eisler et al., 1981; Simpson, 1978; Finberg et al., 1981) which may result in whole or in part from its metabolism to *R*(−)-methamphetamine and *R*(−)-amphetamine (Reynolds et al., 1978). Currently, it is not clear to what extent these metabolites are responsible for the sympathomimetic actions of selegiline since (+)-amphetamine isomers are normally thought to be more pharmacologically active than the (−)-isomers.

Recently, we have described the development of rasagiline [TVP-1012; *R*(+)-AGN1135], a selective irreversible inhibitor of monoamine oxidase-B (Finberg et al., 1996; Youdim et al., 2001). Rasagiline is metabolized to *R*(+)-1-aminoindan and is devoid of sympathomimetic actions in animal experiments (Finberg et al., 1981), presumably because of the minimal sympathomimetic actions of this metabolite, although little is known about the pharmacological actions of aminoindan.

In the present communication, we describe the effects of *R*(−)- and *S*(+)-methamphetamine on sympathetic neurotransmission in rat vas deferens by comparison with *R*(+)- and *S*(−)-1-aminoindan.

2. Materials and methods

2.1. Tissue preparation

The protocol was approved by Technion Animal Care and Use Committee. Male Sprague–Dawley rats (220–300 g) were killed by decapitation. Both vasa deferentia were

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removed and suspended in 30-ml organ baths containing Krebs' solution (mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11) gassed with 95% O_2–5% CO_2 and maintained at 37 °C. The tissues were suspended at an initial tension of 1 g and allowed to stabilize for 60 min, over which period the resting tension reduced to 0.2 ± 0.1 g. Tension was measured isometrically with a Letica (Barcelona, Spain) transducer coupled to a Letica amplifier and recorded on a Graphtec (Tokyo, Japan) pen recorder. The organ bath was equipped with bipolar stimulating electrodes situated above and below the tissue (ring and hook). Pharmacological agents used were noradrenaline bitartrate, yohimbine HCl from Sigma (Rehovot, Israel), *S*(+)-methamphetamine, *R*(−)-methamphetamine, *R*(+)-1-aminoindan, *S*(−)-1-aminoindan from Teva (Petach-Tikva, Israel), and prazosin HCl from Pfizer (Kent, England).

2.2. Twitch responses

Vasa deferentia were field-stimulated with 1-ms square-wave pulses at maximal voltage at a frequency of 0.1 Hz delivered from a Grass S48 stimulator. Following establishment of a stable baseline twitch tension for 15 min, drugs were added to the organ bath every 5 min in increasing concentrations. When response of the twitch tension was maximally depressed by methamphetamine, or after the highest dose of 1-aminoindan, a single dose of yohimbine (260 nM) was administered. In some experiments, prazosin (30 nM) was added to the bath fluid to abolish the action of released noradrenaline on α_1-adrenoceptors. Responses to drugs were determined as the percent of potentiation or inhibition of the isometric twitch tension with respect to initial baseline tension.

2.3. Noradrenaline-induced contractile responses

Noncumulative noradrenaline dose–response curves were constructed in different tissues from those used for electrical field stimulation. Noradrenaline was added in a 5-min cycle (4-min baseline followed by 1-min contact). Following completion of a control dose–response curve, a second set of responses to noradrenaline was obtained in the presence of methamphetamine enantiomers or 1-aminoindan at 0.83 or 8.3 μM. Responses to noradrenaline in the presence of drugs were expressed as percent of maximal response in the control dose–response curve. Additional experiments were carried out to demonstrate the effect of *R*(−)-methamphetamine and cocaine on submaximal responses to noradrenaline and methoxamine.

2.4. Data analysis

Results are expressed as means ± S.E.M. Statistical significance of the observed differences was assessed by repeated measures analysis of variance (ANOVA) or one-way ANOVA as appropriate (SPSS software); EC_{50} and IC_{50} values were calculated by nonlinear regression analysis (Graphpad Prism, Graphpad Software).

3. Results

3.1. Twitch responses

Fig. 1A shows the influence of *S*(+)-, *R*(−)-methamphetamine and *R*(+)-1-aminoindan on the electrically induced twitch response in rat vas deferens. The effect of the methamphetamines was biphasic: potentiation at low concentrations (up to 10^{-6} M) and inhibition at higher concentrations. Yohimbine, added after maximal inhibition of the twitch had been attained by methamphetamine, reversed the inhibitory response, and twitch tension then increased to a value greater than that before addition of the methamphetamines (see Fig. 1). Yohimbine added before methamphetamines prevented the inhibitory response, and only potentiation of the twitch occurred ($n=4$, data not shown). By contrast, *R*(+)- and *S*(−)-1-aminoindan had no effect on the twitch below a concentration of 10^{-5} M and potentiated contractions at concentrations of 10^{-5} and 10^{-4} M [$P<0.01$ for difference from saline control; in Fig. 1, only

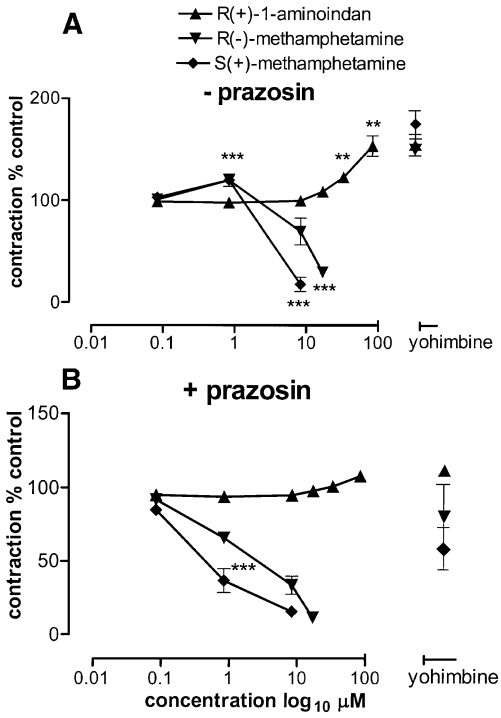

Fig. 1. Potentiation and inhibition of twitch response in rat vas deferens by *S*(+)-methamphetamine, *R*(−)-methamphetamine, and *R*(+)-1-aminoindan. Twitch response to field stimulation (0.1 Hz) in presence of drugs expressed as percent of control twitch response in absence of drugs. (A) In absence of prazosin; $^{**}P<0.01$; $^{***}P<0.001$ for difference from saline control (data not shown); (B) in presence of prazosin (24 nM); $^{***}P<0.001$ for difference between *R*(−)- and *S*(+)-methamphetamine over concentrations 0.1–10 μM. Mean ± S.E.M. for $n=6$ tissues per treatment.

response to *R*(+)-1-aminoindan was shown; identical responses were obtained with *S*(−)-1-aminoindan, $n=4$]. Yohimbine did not modify the response to the higher concentrations of 1-aminoindan enantiomers.

Prazosin abolished the potentiation component of methamphetamine's action and the potentiation produced by *R*(+)- and *S*(−)-1-aminoindan (Fig. 1B). Calculation of IC_{50} values shows that *S*(+)-methamphetamine is 4.6 times more potent in twitch response inhibition than *R*(−)-methamphetamine [$IC_{50}=0.36\pm0.13$, 1.64 ± 0.103 μM, respectively, for *S*(+)- and *R*(−)-methamphetamine; $P<0.01$].

3.2. Noradrenaline-induced contractile responses

Fig. 2A and B shows dose–response curves to noradrenaline in the presence of two concentrations of *S*(+)-, *R*(−)-methamphetamine, and *R*(+)-1-aminoindan [identical responses to those of *R*(+)-1-aminoindan were elicited by *S*(−)-1-aminoindan, $n=4$]. At a concentration of 0.83 μM, both methamphetamines produced an equivalent increase in the potency of noradrenaline by a factor of 4.7-fold [EC_{50} for control, *R*-(+)- and *S*-(−)-methamphetamine $=3.56\pm0.09$, 0.78 ± 0.09, and 0.72 ± 0.18 μM, respectively; $P<0.01$ for difference between methamphetamines and control; $P>0.05$ for difference between methamphetamines]. When administered at a concentration of 8.3 μM, sensitivity to noradrenaline was increased by a factor of 10 for both methamphetamines [EC_{50} for control, *R*-(+)- and *S*-(−)-methamphetamine $=4.21\pm0.064$, 0.35 ± 0.20, and 0.43 ± 0.15 μM, respectively; $P<0.01$ for difference between methamphetamines and control; $P>0.05$ for difference between methamphetamines]. *R*(+)- and (*S*)-1-aminoindan did not significantly affect EC_{50} to noradrenaline at either 0.83 or 8.3 μM. Contractile responses to a submaximal concentration of methoxamine (1 μM) were not potentiated by methamphetamine (5 μM). The potentiation of a submaximal response to noradrenaline (1 μM) caused by (−)-methamphetamine (5 μM) was prevented by cocaine (30 μM; Fig. 2).

4. Discussion

Amphetamines can affect the sympathetic neuron by three major mechanisms: (1) non-exocytotic release of noradrenaline, (2) reversible inhibition of monoamine oxidase-A, and (3) inhibition of the plasma-membrane noradrenaline transporter (U_1). Noradrenaline released following amphetamine administration will interact with postsynaptic excitatory α_1- and presynaptic inhibitory α_2-adrenoceptors. The response to a single electrical depolarising stimulation consists of two components: the "fast" one, induced by ATP, and the "slow" one, induced by noradrenaline (McGrath, 1978; Lundberg, 1996). When stimuli are given at frequencies of 0.1 Hz, as in

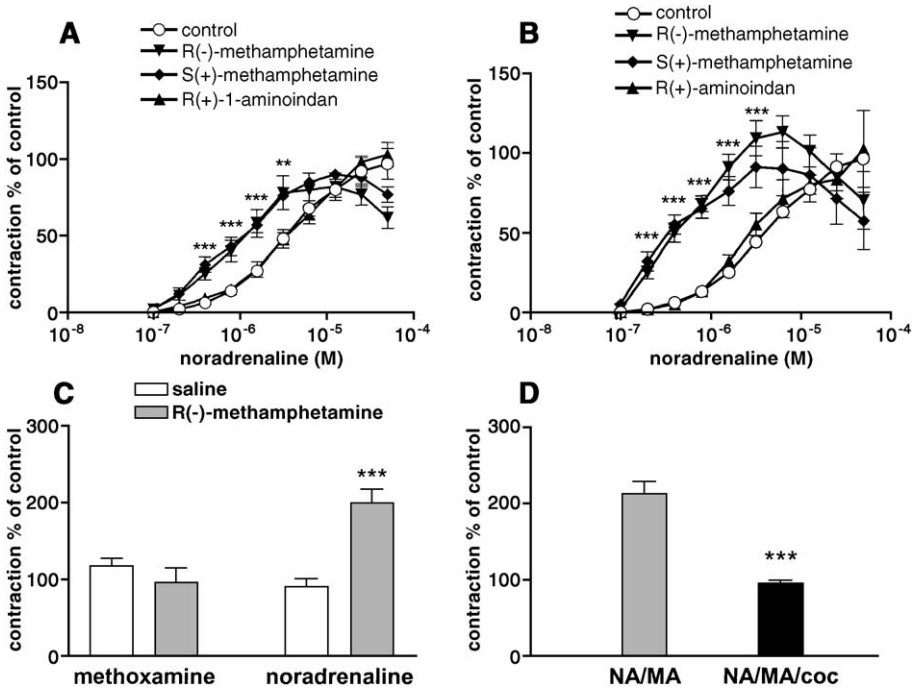

Fig. 2. Modification of agonist-induced contractile response of rat vas deferens by methamphetamine and aminoindan. (A–B) Dose response to noradrenaline in presence of methamphetamine enantiomers and 1-aminoindan at concentrations: (A) 0.83 μM; (B) 8.3 μM. Contractile response to noradrenaline expressed as percent of maximal response obtained in initial dose–response curve in absence of drugs. ***$P<0.001$ and **$P<0.01$, for difference between means of both methamphetamine enantiomers and control. (C) Potentiation of submaximal contractile responses to methoxamine (2 μM) and noradrenaline (1 μM) by *R*(−)-methamphetamine (5 μM); (D) potentiation of noradrenaline-induced (NA; 1 μM) contractile response by *R*(−)-methamphetamine (MA; 5 μM) with and without cocaine (coc; 30 μM). Mean ± S.E.M. for $n=5–6$ tissues per drug treatment or 15–16 control tissues. ***$P<0.001$ for difference from control.

the current experiments, the slow component of contraction disappears, and only the fast component is seen (Mallard et al., 1992).

The depressant effects of methamphetamine on electrically induced twitch response presented here are similar to those described by Urabe et al. (1987) and are presumed to be mediated by released noradrenaline since amphetamines, in general, have no direct agonist activity at the α_1-adrenoceptor (Weiner, 1985). At low concentrations, this released noradrenaline activates the postsynaptic α_1-adrenoceptors to an extent which by itself is not sufficient to cause contraction, but enhances the ATP-induced contraction, by increased influx of Ca^{2+} into the postsynaptic cell (Sakai et al., 1989; Lundberg, 1996). At higher concentrations, activation of presynaptic α_2-adrenoceptors by released noradrenaline effectively inhibits release of both neurotransmitters, resulting in blockade of twitch contraction. The reversal of this inhibition by yohimbine demonstrates the involvement of presynaptic α_2-adrenoceptors in the inhibitory response. At still higher concentrations, amphetamines can cause depolarisation-induced contraction of vascular and other smooth muscle (Weiner, 1985).

Prazosin converted the biphasic response to methamphetamines to an inhibitory response, as well as antagonising the twitch-potentiating effect of 1-aminoindan, indicating that the twitch potentiation is probably mediated by released noradrenaline acting at α_1-adrenoceptors.

Both methamphetamine stereoisomers increased the potency of exogenous noradrenaline in inducing isometric contraction of vas deferens, which could result both from inhibition of uptake-1 and from postsynaptic potentiation as described above. The prevention of methamphetamine-induced potentiation of the noradrenaline response by cocaine, however, together with the lack of potentiation of methoxamine-induced contraction, shows that the most likely mechanism for this potentiation was inhibition of uptake-1 (methoxamine is not a substrate for uptake-1; Al-Damluji et al., 1993). On the contrary, neither *R*(+)- nor *S*(−)-1-aminoindan increased the potency of noradrenaline at the concentrations studied (0.83, 8.3 μM), consistent with the reported weak effectiveness of 1-aminoindan on amine uptake (Horn and Snyder, 1972). *S*(+)-Methamphetamine is about 10 times more potent than *R*(−)-methamphetamine on eliciting release of striatal dopamine and dopaminergic behavioural responses (Melega et al., 1999); yet, in inducing noradrenaline release in the CNS, the isomers have similar activity (Kuczenski et al.,1995).

The slightly greater effectiveness of *S*(+)-methamphetamine in inhibition of the twitch response may be the result of the more complex character of this response, which involves also exocytotic release mechanisms as well as an action on the noradrenaline transporter.

These observations support the possibility of a role of *R*(−)-methamphetamine in mediation of the sympathomimetic effects of selegiline. The very weak sympathomimetic effects of *R*(+)-1-aminoindan are consistent with the lack of sympathomimetic action of rasagiline in animal experiments (Finberg et al., 1981). Since the dose of rasagiline in humans is in the region of one-fifth that of selegiline, sympathomimetic effects of this drug in humans are unlikely.

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