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Effects of Clonidine and α-Adrenoceptor Antagonists on Motor Activity in DSP4-Treated Mice II: Interactions with Apomorphine

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Adult mice were administered either the noradrenaline (NA) neurotoxin, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine, (DSP4), or distilled water (control), 10–12 days before motor activity testing, and 6 h before testing all the mice were administered reserpine (10 mg/kg), the monoamine-depleting agent. The interactive effects of (I) clonidine, the α_2-adrenoceptor agonist, with the dopamine (DA) agonist, apomorphine, and the α_2-antagonist, yohimbine, and (II) with either yohimbine or the α_1-antagonist, prazosin, upon motor behaviour in activity test chambers were studied in reserpinized DSP4-treated and control mice. It was shown that apomorphine (3 mg/kg) increased locomotor and total activity in both reserpinized DSP4-treated and control mice but the effect was attenuated in the DSP4 mice. Co-administration of clonidine (3 mg/kg) with apomorphine potentiated the effects of apomorphine on motor activity and this effect was enhanced markedly by DSP4 pretreatment. Yohimbine (10 mg/kg) antagonised the motor activity-stimulating effects of apomorphine in both DSP4-treated and control mice. Co-administration of clonidine with apomorphine, following yohimbine, restored motor activity levels to those obtained in the absence of yohimbine and this effect upon locomotor activity was enhanced by DSP4 pretreatment. The effects of clonidine on motor activity were enhanced by NA-denervation. Prazosin (3 mg/kg) enhanced the locomotor activity of both reserpinized DSP4-treated and control mice after the initial 30-mm period but was not affected by DSP4 treatment. Analysis of post-decapitation convulsions (PDCs) indicated loss of the reflex by DSP4 pretreatment. Reserpine pretreatment abolished the initial, exploratory phase (30 min) of motor activity. These results demonstrate interactions between NA and DA systems that may bear eventual relevance to neurologic disorders such as parkinsonism.

Keywords: DSP4, Control, Reserpine, Locomotion, Total activity, Clonidine, Apomorphine, Co-administration, Enhancement, Yohimbine, Antagonism, Prazosin, Supersensitivity, Attenuation, PDCs, Movement disorders, Mice

INTRODUCTION

Possible interactions between noradrenergic and dopaminergic pathways in the mediation of motor behaviour have been considered for some time (e.g. Archer *et al.*, 1986; Ögren *et al.*, 1983) and recent clinical evidence underlines the interaction in Parkinson's disease (Riekkinen *et al.*, 1998). Goldstein *et al.* (1983) outlined a possible

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regulation of dopamine (DA) neurotransmission by the noradrenaline (NA) pathways presenting evidence that clonidine, an α_2-adrenoceptor agonist, may interact with presynaptic DA receptors. Carlsson and Carlsson (1989) pretreated mice with reserpine, that depletes brain and CNS stores of monoamines, and α-methyl-p-tyrosine, the catecholamine synthesis inhibitor, and found a synergistic interaction of clonidine with the non-competitive glutamate antagonist, MK-801, upon locomotor activity. These interactive synergistic effects were antagonised by the α_2-adrenoceptor antagonists, yohimbine and idazoxan. Other studies extended these findings of synergistic interactions between α-adrenoceptors and glutamate antagonists in motor activity (Carlsson and Svensson, 1990a,b; Carlsson *et al.*, 1991). Non-competitive glutamate antagonists induce similar behaviour effects, e.g. locomotion and stereotypy, in a dose-dependent manner similar to apomorphine (see Schmidt, 1994), and these stimulatory effects are antagonised in a similar manner by neuroleptic compounds (Ögren and Goldstein, 1994).

The selective neurotoxic properties of the compound, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4), for NA neurons in the forebrain, midbrain, cerebellum, brain stem and spinal cord have been extensively described (Jonsson, 1980; Jonsson and Hallman, 1982; Jonsson *et al.*, 1981; 1982; Ross, 1976; Ross and Renyi, 1976). Systemic administration of higher doses of DSP4 (50 mg/kg, i.p. and above) produced profound decreases in dopamine-β-hydroxylase (Ross and Renyi, 1976) and induced marked and permanent reductions of endogenous NA in the cerebral and cerebellar cortex, hippocampus and spinal cord, leaving DA and serotonin (5-HT) neurons apparently unaffected (Jonsson *et al.*, 1981), although a small but significant reduction of 5-HT has sometimes, but not always, been reported (Jonsson *et al.*, 1982). Lower doses (3 and 6 mg/kg, i.p.) produced small but significant decreases in NA concentrations in some brain regions of rats, e.g. cerebral cortex, hippocampus, olfactory bulb and spinal cord (Archer *et al.*, 1984).

The purpose of this study was to examine the effects of clonidine, by itself, and in co-administration with apomorphine upon motor activity in monoamine-depleted mice that either had an intact noradrenergic system or had undergone NA-denervation through pretreatment with DSP4. In order to ascertain the involvement of α-adrenoceptor sites the effects of yohimbine and prazosin, α_2 and α_1 receptor antagonists respectively, upon these interactions was examined. Since clonidine, yohimbine and prazosin all possess some presynaptic activity the removal of presynaptic NA terminals by DSP4 should have induced α-adrenoceptor adaptive changes.

MATERIALS AND METHODS

Animals

In all the three experiments described, six-month-old male C57BL/6 mice (ALAB, Sollentuna, Sweden), weighing 28–30 g were used. Following arrival at the laboratory, the mice were allowed to acclimatise for two weeks in a room with controlled temperature (21 ± 1°C), and a constant light–dark schedule (12 h on/12 h off, lights on between 06:00 and 18:00 h). Free access to food and water was maintained throughout. They were housed in groups of 10 animals and tested only during the hours of light (08:00–15:00 h). All testing was performed in a normally lighted room. This test room, in which all 12 ADEA activity test chambers, each identical to the home cage, were placed, was well-secluded and used only for this purpose. Each test chamber (i.e. activity cage) was placed in a sound-proofed wooden box with 12 cm thick walls and front panels and had a dimmed lighting. Experiments were carried out in accordance with the European Communites Council Directive of 24 November 1986 (86/609/EEC) after approval from the local ethical committee (Uppsala University and Agricultural Research Council).

Behavioural Measurements and Apparatus

An automated device, consisting of macrolon rodent test cages (40 × 25 × 15 cm) each placed within two series of infrared beams (at two different heights, one low and one high, 2 and 8 cm, respectively, above the surface of the sawdust, 1 cm deep), was used to measure spontaneous and/or drug-induced motor activity of MPTP and control mice (RAT-O-MATIC, ADEA Elektronic AB, Uppsala, Sweden). According to the procedures described previously (Archer *et al.*, 1986; Fredriksson and Archer, 1994), the following parameters were measured: Locomotion, rearing and total activity. However, in this study only the locomotion and total activity data were of utility: Locomotion counts were measured by the low level grid of infra-red beams. Counts were registered only when the mouse moved in the horizontal plane, ambulating around the test cage. Total activity counts were measured by a sensor (a pick-up similar to a gramophone needle, mounted on a lever with a counterweight) with which the test cage was in constant contact. The sensor registered all types of vibration received from the test cage, such as those produced both by locomotion and rearing as well as shaking, tremors, scratching and grooming. All three behavioural parameters were measured over either three or four consecutive 30-min periods.

Treatment and Chemicals

DSP4 (synthetised by Astra Arcus AB, Södertälje, Sweden) was injected intraperitoneally to half of the mice in each experiment at a single dose of 75 mg/kg, the other half of mice received distilled water (control). DSP4 was dissolved in distilled water. Reserpine, apomorphine, clonidine, yohimbine and prazosin (gifts from Astra Arcus AB, Sodertalje, Sweden) were dissolved in physiological saline (0.9%). All solutions (saline was used as vehicle) were injected subcutaneously in a volume of 5 ml/kg, s.c. Dosages are expressed as the free base.

Experimental Design and Procedure

Experiment I

Six groups were treated with DSP4 (75 mg/kg, i.p.) and six groups ($n = 12$) with distilled water (control) 10–12 days prior to behavioural testing. Six hours before testing all the groups were administered reserpine (10 mg/kg) and each animal replaced in its home cage. At testing, two DSP4-treated groups (DSP-yoh-apo and DSP-yoh-apo + clon) and two control groups (CON-yoh-apo and CON-yoh-apo + clon) were administered yohimbine (10 mg/kg, s.c.) followed 15 min later by either apomorphine (3 mg/kg, s.c.) and saline (2 ml/kg) or apomorphine and clonidine (3 mg/kg, s.c.) and then placed immediately in the test chambers. Four DSP4-treated groups (DSP-apo, DSP-clon, DSP-apo + clon and DSP-sal) and four control groups (CON-apo, CON-clon, CON-apo + clon and CON-sal) were administered saline 15 min before the appropriate injections of either apomorphine (3 mg/kg), clonidine (3 mg/kg), apomorphine (3 mg/kg) + clonidine (3 mg/kg) or saline and then placed immediately in the motor activity test chambers. Motor activity was then registered over four 30 min periods (0–30, 30–60, 60–90 and 90–120 min), but for data analysis the 1st 30-min period and the mean of the 2nd, 3rd and 4th periods were used.

Experiment II

Six groups ($n = 12$) were treated with DSP4 (75 mg/kg) and six groups ($n = 12$) with distilled water 10–12 days prior to behavioural testing. Six hours before testing all 12 groups were administered reserpine (10 mg/kg) and then replaced in their home cages. At testing, two DSP4-treated groups (DSP-yoh-clon and DSP-yoh-sal) and two control groups (CON-yoh-clon and CON-yoh-sal) were administered yohimbine (10 mg/kg) followed 15 min later by either clonidine (3 mg/kg) or saline (2 ml/kg), respectively. Two DSP4-treated groups (DSP-pra-clon and DSP-pra-sal)

and two control groups (CON-pra-clon and CON-pra-sal) were administered prazosin (1 mg/kg, i.p.) followed 15 min later by clonidine (3 mg/kg) or saline, respectively. Two DSP4-treated groups (DSP-sal-clon and DSP-sal-sal) and two control groups (CON-sal-clon and CON-sal-sal) were administered saline followed 15 min later by either clonidine (3 mg/kg) or saline, respectively. Immediately after the respective clonidine and saline injections each mouse was placed in its motor activity test chamber. Motor activity counts were then registered over four 30 min test periods (0–30, 30–60, 60–90 and 90–120 min), but for data analysis the 1st 30-min period and the mean of the 2nd, 3rd and 4th periods were used.

Statistical Analysis

In both experiments, it was evident that activity counts between groups varied considerably from the first to the second 30 min but not with each ensuing 30-min period. Therefore locomotion and rearing counts for each mouse during the second-fourth periods were summated and divided by three to provide mean counts during the whole 90 min. These mean count data from the first and second periods were subjected to a one-way ANOVA based on a completely randomised design (cf. Kirk, 1995). Pairwise differences between groups were tested for using Tukey's HSD tests. The 1% level of significance was maintained throughout.

RESULTS

Experiment I

Apomorphine (3.0 mg/kg) increased the motor activity of both DSP4-treated and control reserpinized mice; the effect was markedly attenuated in the DSP4-treated mice. The stimulatory effect of apomorphine was potentiated by the co-administration of clonidine (3.0 mg/kg); this effect was markedly enhanced in the DSP4-treated mice. Yohimbine (10 mg/kg) antagonised the stimulatory effects of apomorphine in the control mice and this effect was almost completely abolished in the DSP4-treated mice. Clonidine reversed the antagonistic effects of yohimbine, upon apomorphine, in both DSP4-treated and control mice. For locomotor behaviour, and to a much lesser extent total activity, the stimulatory effects of apomorphine, clonidine and yohimbine co-administration were enhanced in the DSP4-treated mice. During the 0–30 min period, total activity counts by the yohimbine + apomorphine group were elevated in DSP4-treated mice. Thus, split-plot ANOVA indicated Groups × Time periods effects for both locomotion: $F(11, 132) = 13.02$, and total activity: $F(11, 132) = 9.88$, respectively. Figure 1 presents the locomotor and total activity counts by DSP4-treated and control reserpinized mice administered apomorphine and clonidine, by themselves or in combination, or together with yohimbine. Tukey's HSD tests indicated the pairwise differences outlined in Table I.

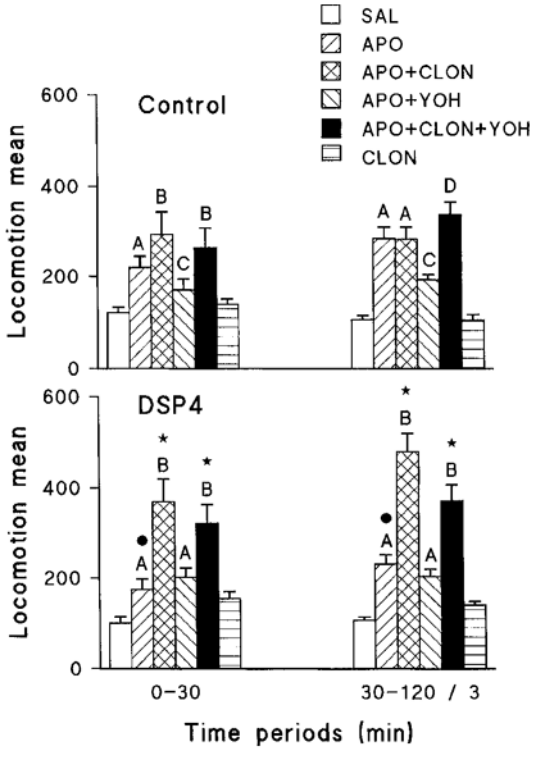

FIGURE 1(a)

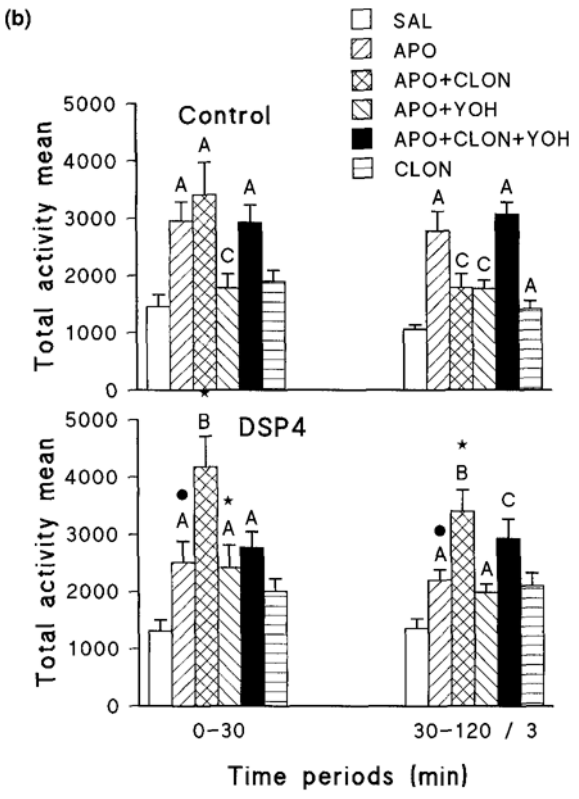

FIGURE 1(b)

FIGURE 1 The effects of clonidine (3 mg/kg), apomorphine (3 mg/kg), apomorphine + clonidine, yohimbine (10 mg/kg) + apomorphine, or yohimbine + apomorphine + clonidine, upon motor activity in reserpinized DSP4-treated and control mice. Mean locomotion (a) and total activity (b) counts during the 1st 30-min period and the mean of the following 2nd, 3rd and 4th 30-min periods. DSP4 (75 mg/kg) or distilled water (control) was administered 10–12 days before testing; reserpine (10 mg/kg) was administered to all the mice 6 h before behavioural testing. Yohimbine was administered 15 min before clonidine/apomorphine. Values represent means ± SD of 12 mice. $^{A}p < 0.01$, versus SAL, $^{B}p < 0.01$, versus APO, $^{C}p < 0.01$, less than APO, Tukey HSD tests; $^{*}p < 0.01$, enhanced versus respective control group, Tukey HSD tests; $^{\bullet}p < 0.01$, reduced versus respective control group, Tukey HSD tests.

TABLE I Significant differences obtained from pairwise comparisons, using Tukey's HSD test, between reserpinized DSP4-treated and control mice administered either clonidine or apomorphine, or both, following injection or either yohimbine or prasozin or saline 15 min previously in Experiments I and II

Experiment I
Locomotion
Control
0–30: apo + clon, yoh-apo + clon > apo > yoh-apo > sal, clon
30–120: yoh-apo + clon > apo + clon, apo > yoh-apo > sal, clon
DSP4-treated
0–30: apo + clon, yoh-apo + clon > yoh-apo, apo > clon, sal
30–120: apo + clon > yoh-apo + clon > apo, yoh-apo > clon, sal
Total activity
Control
0–30: apo + clon, apo, yoh-apo + clon > yoh-apo > clon, sal
30–120: yoh-apo + clon, apo > apo + clon, yoh-apo > clon > sal
DSP4-treated
0–30: apo + clon > yoh-apo + clon, apo, yoh-apo > clon, sal
30–120: apo + clon > yoh-apo + clon > apo, yoh-apo > clon, sal
Experiment II
Locomotion
Control
0–30: sal, clon, praz + clon, praz, yoh + clon > yoh
30–120: yoh + clon > praz + clon, praz, clon > sal > yoh
DSP4-treated
0–30: clon > sal > yoh; praz + clon > praz > yoh; clon > yoh + clon > yoh
30–120: praz + clon, clon, praz, yoh + clon > sal > yoh
Total activity
Control
0–30: clon > yoh
30–120: yoh + clon > clon > yoh, sal; praz + clon, praz, clon > sal
DSP4-treated
0–30: clon, praz + clon > yoh + clon, sal, praz > yoh
30–120: praz + clon > clon, yoh + clon, praz > yoh, sal
The 1% level of significance was maintained throughout.

The following differences were obtained due to the DSP4 pretreatment:

(1) The stimulatory effects of the apo + clon and yoh-apo + clon groups upon locomotor activity were potentiated significantly.
(2) The stimulatory effects of apo + clon upon total activity were potentiated significantly.
(3) The stimulatory effects of apo by itself upon both locomotor and total activity were reduced significantly.
(4) The effects of yoh-apo upon total activity were enhanced during the first (0–30 min) period.

Experiment II

Clonidine administration to reserpinized mice enhanced motor activity in the DSP4 pretreated animals: Yohimbine (10 mg/kg) by itself reduced the locomotor and total activity of both reserpinized DSP4-treated and control mice. Co-administration of yohimbine and clonidine antagonised (0–30 min) and reversed (30–120 min) the suppressant effects of yohimbine. Prasozin, on the other hand, increased motor activity during the 30–120 min period. Co-administration of prazosin and clonidine enhanced total activity further in DSP4-treated but not control mice. The effects of clonidine (0–30 min) were enhanced whereas those of yohimbine were reduced by DSP4 pretreatment. Finally, the activity-elevating effects of the yohimbine + clonidine combination (30–120 min) were reduced by DSP4. Thus, split-plot ANOVA indicated significant Groups × Time periods effects for locomotion: $F(11, 132) = 56.82$, as well as total activity: $F(11, 132) = 34.64$, respectively. Figure 2 presents the locomotor and total activity counts of reserpinized DSP4-treated and control mice administered clonidine, yohimbine and prasozin by themselves or in combination, yohimbine + clonidine or prazosin + clonidine.

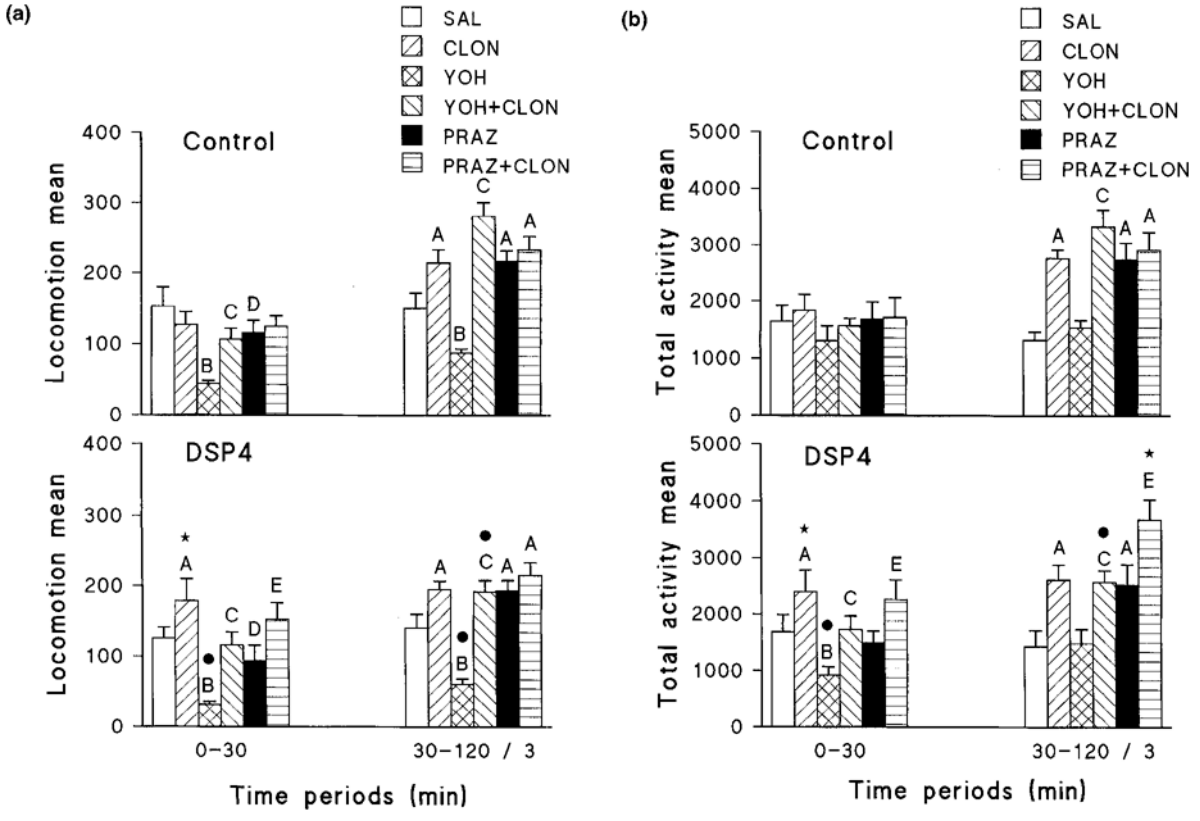

FIGURE 2 The effects of clonidine (3 mg/kg), yohimbine (3 mg/kg), yohimbine + clonidine, prazosin (3 mg/kg), prazosin + clonidine, upon motor activity in reserpinized DSP4-treated and control mice. Mean locomotion (a) and total activity (b) counts during the 1st 30-min period and the mean of the following 2nd, 3rd and 4th 30-min periods. DSP4 (75 mg/kg) or distilled water (control) was administered 10–12 days before testing; reserpine (10 mg/kg) was administered to all the mice 6 h before behavioural testing. Yohimbine was administered 15 min before clonidine/apomorphine. Values represent means ± SD of 12 mice. $^{A}p < 0.01$, versus SAL, $^{B}p < 0.01$, less than SAL, $^{C}p < 0.01$, versus YOH, Tukey HSD tests; $^{*}p < 0.01$, enhanced versus respective control group, Tukey HSD tests; $^{\bullet}p < 0.01$, reduced versus respective control group, Tukey HSD tests.

Pairwise differences between groups were tested for using Tukey's HSD test and are presented in Table I.

The following significant differences were obtained due to DSP4 pretreatment:

(1) The effect of clon on locomotor and total activity was enhanced during the first period.
(2) The suppressant effect of yoh was further reduced (except total activity, second period).
(3) The effect of praz + clon on total activity was enhanced.

Analysis of Post-Decapitation Convulsions

Post-decapitation convulsion ratings were assessed on a scale of 0 to +++, as described previously (Archer *et al.*, 1982; 1984; 1985). As shown in previous studies the loss of decapitation seizures which is a measure of degeneration of noradrenergic nerves in the spinal cord (Mason and Fibiger, 1979; Roberts *et al.*, 1978) was complete with the dose (75 mg/kg) of DSP4 applied throughout.

DISCUSSION

In the present study, the interactive effects of the α_2-agonist, clonidine, (I) with the DA agonist, apomorphine, and the α_2-antagonist, yohimbine, and (II) with either yohimbine or the α_1-antagonist, prazosin, were studied in reserpinized DSP4-treated and control mice. The results may be summarised as follows: (1) Apomorphine (3 mg/kg) markedly increased locomotor and total activity in both reserpinized DSP4-treated and control mice during the whole 120-min period of motor activity testing; this effect was attenuated in DSP4-treated mice. (2) Co-administration of clonidine (3 mg/kg) with apomorphine potentiated locomotor behaviour during the first 30 min of testing and total activity during the whole 120-min test period in the control mice; pretreatment with DSP4 (75 mg/kg, 10–12 days earlier) enhanced substantially the motor activity counts, particularly the locomotor counts, of reserpinized mice co-administered clonidine and apomorphine. (3) Yohimbine (10 mg/kg), injected 15 min before apomorphine, antagonised the motor activity-increasing effects of apomorphine in both DSP4-treated and control mice; note that for total activity this effect was considerably attenuated by DSP4 pretreatment. (4) Co-administration of clonidine together with apomorphine, 15 min after yohimbine treatment, restored motor activity levels to those obtained in the absence of yohimbine; this restoration effect was enhanced for locomotor behaviour in the NA-denervated mice. (5) The effects clonidine (3 mg/kg) on motor activity during the first period were enhanced in reserpinized DSP4-treated mice. The stimulatory effects of clonidine during the second period were potentiated by pretreatment with yohimbine (10 mg/kg) in control but not DSP4 mice. (6) Pretreatment with prazosin (3 mg/kg) elevated locomotor activity in both DSP4-treated and control mice after the initial 30-min period. DSP4 pretreatment enhanced the total activity counts of mice co-administered prazosin and clonidine. (7) Analysis of post-decapitation convulsions indicated reliable loss of the reflex in the mice treated earlier with DSP4. Ancilliary to the main findings it was found that (a) pretreatment with reserpine (10 mg/kg, 6 h before testing) abolished the initial, exploratory phase of motor activity in the first 30 min of testing, (b) prazosin (3 mg/kg), by itself, increased locomotor and total activity counts (during the second period) in both reserpinized DSP4-treated and control mice whereas yohimbine (3 mg/kg) reduced locomotor counts but only affected total activity counts in DSP4 mice during the first period. It must be borne in mind that the 10 mg/kg dose of reserpine used affects thermoregulation which may have influenced the results, and although this consideration applies to several other studies of DA–NA interaction effects the interaction with DSP4 pretreatment in that regard is not known.

α-Adrenergic and dopaminergic interactions have been evidenced following depletions of brain monoamines (Dolphin *et al.*, 1976). For example, Starr and Starr (1994) found that clonidine enhanced profoundly the motor responses to RU 24213, a DA D_2 agonist, and apomorphine but not SKF 38393, a DA D_1 agonist, in mice rendered akinetic by pretreatment with reserpine 24 h earlier. Eshel *et al.* (1990) pretreated mice with reserpine and α-methyl-p-tyrosine to deplete monamines and inhibit catecholamine (CA) synthesis and observed that clonidine potentiated apomorphine-induced locomotor stimulation; clonidine potentiated also locomotor stimulation induced by quinperole, a mixed DA D_2/D_3 receptor agonist, an effect antagonised by prazosin but not yohimbine. The authors concluded that an α_1-receptor agonist in combination with DA D_2 agonists produces marked stimulation in DA (CA) depleted mice (Eshel *et al.*, 1990). In Experiment I, the potentiated effects of clonidine + apomorphine were antagonised by yohimbine in *DSP4-treated mice, 10–12 days previously,* a denervating procedure combined with the reserpine-depleting as opposed to only the depleting procedure of Eshel *et al.* (1990), thereby involving post-synaptic adrenoceptor changes. Prazosin was not tested here in that combination: nevertheless, in DA-depleted MPTP-treated mice yohimbine *and not prazosin* was found to antagonise the psychostimulatory effects of clonidine + L-Dopa (Archer and Fredriksson, manuscript submitted to Neurotoxicity Research, Special Issue, 2000). Henry *et al.* (1998) indicated that the dyskinesias induced by repeated administration of L-Dopa to rats with unilateral 6-hydroxydopamine lesions that performed rotational behaviour could be abolished by yohimbine. Pucilowski *et al.* (1987) injected DSP4 into the central amygdala that caused a marked and selective NA depletion (29% of control) and found a significant increase in both the induction of locomotor activity and aggressive behaviour by apomorphine (5 mg/kg). The present results confirm this interaction in reserpinized DSP4-treated and control mice in several ways: (1) Motor activity (locomotor and total) induced by acute apomorphine administration during the initial 30 min of testing was reduced by NA depletion. (2) Co-administration of clonidine with apomorphine enhanced the motor activity induced by apomorphine, particularly during the first 30 min, and this enhancement was markedly increased for locomotor behaviour in NA-denervated DSP4-treated mice. (3) Yohimbine pretreatment, before apomorphine, antagonised effectively the activity-inducing actions of apomorphine in control mice but either did so to a lesser extent or not at all in DSP4-treated mice. (4) Combination of clonidine with apomorphine following yohimbine pretreatment restored the motor activity to levels comparable to clonidine plus apomorphine without yohimbine pretreatment in control but not DSP4-treated mice, that showed a less complete restoration. Thus, it appears that yohimbine fails to antagonise the potentiation of apomorphine-induced activity by clonidine in reserpinized control mice but attenuated this effect in reserpinized DSP4-treated mice, implying a post-synaptic site. It is possible that the effects of yohimbine upon NA release from presynaptic terminals may be involved in the lack of effect seen in the reserpinized control mice. Since the locomotor activity-inducing effects of clonidine, by itself, were minimal in DSP4-treated mice and the same effects of apomorphine only moderate, a synergistic effect of the combination (cf. Carlsson and Carlsson, 1989) is suggested; in view of the presynaptic terminal denervation induced by DSP4, it is likely that the observed synergism involved a supersensitive postsynaptic α-adrenoceptor-mediated effect. Thus, it seems likely that the interaction between α_2-receptors and DA occurs at postsynaptic sites.

Several studies have indicated that lesions to noradrenergic systems enhance the extent of destruction to DA neurons and depletion of forebrain DA. For instance, Marien *et al.* (1993) pretreated C57BL/6 mice with DSP4 (40 mg/kg) before administering MPTP (4×10 mg/kg over 8 h)

and measured striatal DA levels seven days later. DA depletions were 60% whereas in the absence of prior DSP4 they were 40%. Fornai *et al.* (1997) administered DSP4 (50 mg/kg) either 12 h before or after administration of the selective DA neurotoxin, MPTP (30 mg/kg), and found that only administration of DSP4 before MPTP enhanced the DA depletion, suggesting a more pronounced acute neuronal sensitivity to MPTP occurs in NA-depleted mice. This effect was independent of modifications in the striatal kinetics of MPTP/MPP^+ measured at seven different time intervals after MPTP administration. The enhancement of DA-depletion following DSP4-induced loss of NA extended also methamphetamine-induced, moderate (10–20 mg/kg) dose, partial striatal DA-depletion seven days after drug administration in both rats and mice (Fornai *et al.*, 1998a,b). A low dose (1×5 mg/kg and 3×5 mg/kg, respectively, i.p., at 2-h intervals) of methamphetamine that did not reduce striatal DA produced significant reductions when administered to DSP4-pretreated Sprague–Dawley rats and C57Bl/6N mice (Fornai *et al.*, 1996). Clonidine enhanced methamphetamine toxicity whereas α_2-antagonists reduced it. It was shown further that a more pronounced acute neuronal sensitivity to methamphetamine also occurs in DSP4-treated Swiss–Webster mice (Fornai *et al.*, 1999). Of relevance to the present findings, NA infusion induced a decrease, whereas yohimbine induced an increase, in plasma DA (Musso *et al.*, 1992). DSP4 by itself appears to produce negligible effects upon NA in the DA-rich areas of the brain: NA-innervation in the nigrostriatal and mesolimbic DA systems appear mostly unaffected by DSP4 in rats; thus, DSP4 (50 mg/kg, standard dose) only slightly reduced NA concentrations in either the striatum or nucleus accumbens, whereas NA levels in the A9 and A10 areas were reduced by between 20% and 40% (Archer *et al.*, 1984).

Pretreatment with yohimbine potentiated the small increase in locomotor activity induced by clonidine in reserpinized control mice and this effect was attenuated by DSP4-induced NA-denervation. However, pretreatment with yohimbine antagonised the marked increase in total activity induced by clonidine and this effect was also attenuated (i.e. the separation between the two groups) by DSP4 treatment, probably due to the significant antagonism of the stimulatory effects of clonidine by pretreatment with the neurotoxin. On the one hand, it may be argued that a presynaptic-releasing action of any residual NA at the nerve terminals of reserpinized control mice by yohimbine may be responsible and the prior administration of DSP4 removed those terminals but this cannot explain the antagonistic action of yohimbine upon clonidine-induced total activity; here, perhaps the putative actions of clonidine at presynaptic receptors and/or α_1-adrenoceptors may be invoked (Eshel *et al.*, 1990). Furthermore, Chopin *et al.* (1986) attributed the antagonistic actions of yohimbine and clonidine upon each other to their α_2-sites of action, as indicated in other circumstances (Kostowski and Malatynska, 1983; Kunchandy and Kulkarni, 1986; Nishikawa *et al.*, 1983). Prazosin, α_1-adrenoceptor antagonist, increased locomotor and total activity, by itself, in reserpinized DSP4-treated and control mice, but did not affect clonidine-induced locomotor activity. For total activity, prazosin did not affect (i.e. antagonise) the stimulatory effects of clonidine in control mice but in the NA-denervated DSP4-treated mice the number of total activity counts was significantly enhanced by the co-administration of prazosin and clonidine both compared to the same treatment in control mice and the DSP4 mice that received prazosin. Taken together, yohimbine antagonised the functional effect of clonidine, whereas prazosin potentiated it (Experiment II); the antagonistic effect of yohimbine was attenuated by pretreatment with DSP4, whereas the potentiation by prazosin was enhanced. These observations seem to confirm the somewhat conflicting actions of each compound in relation to clonidine. The involvement of presynaptically-mediated α_2–α_1-adrenoceptor site mechanisms,

respectively, in the actions of yohimbine and prazosin (cf. Biaggioni *et al.*, 1994; Cristofol and Rodriguez-Farre, 1991; Sanchez-Merino *et al.*, 1990) or that of α_2A and α_2B adrenoceptors at presynaptic sites (Gobbi *et al.*, 1990; 1993; Limberger *et al.*, 1991) ought to be implicated. Further, some evidence suggests that hippocampal and cortical NA release is regulated by α_2D-adrenoceptors, a species variation of the human α_2A-subtype (Kiss *et al.*, 1995). It ought to be indicated that the effects seen above may in part be mediated by an action on an imidazoline receptor subtype: recently, Meana *et al.* (1997) studied the effects of imidazoli(di)ne derivatives, e.g. clonidine, upon the extracellular release of NA, DA, DOPAC and HVA in the cingulate cortex using microdialysis. Clonidine induced a dose-dependent (0.3–1.2 mg/kg, i.p.) decrease in NA and DA that was reversed by the α_2-antagonist, RX821002, possibly involved in the synergistic effects of combining apomorphine in DSP4-treated mice, particularly.

The present findings demonstrate a notable noradrenergic–dopaminergic interaction in monoamine-depleted, NA-denervated mice that bear some relevance for and confirm the previous indications of the role of α-adrenoceptors in parkinsonism (e.g. Berlan *et al.*, 1989; Cash *et al.*, 1984; Riekkinen *et al.*, 1998), despite possible neurological side-effects of the acute treatments involved. Thus, the interactive effects of clonidine with apomorphine bear out the observed anti-parkinsonian effects of the former in the clinic (Shoulson and Chase, 1976; Tarsy *et al.*, 1975).

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