Naunyn-Schmiedeberg's Arch Pharmacol (1991) 343:568–573
002812989100088N

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Synergistic interactions between muscarinic antagonists, adrenergic agonists and NMDA antagonists with respect to locomotor stimulatory effects in monoamine-depleted mice

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Received September 26, 1990/Accepted March 5, 1991

Summary. The purpose of the present investigation was to study the effects of simultaneous manipulations of central cholinergic, adrenergic and glutamatergic systems on locomotion in an animal model of Parkinson's disease.

Mice were deprived of their monoamine stores by pretreatment with the monoamine depleter reserpine and the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine, given 18 h and 60 min, respectively, before the acute experiment. Traditionally, only dopaminergic agonists have been shown to reverse the akinesia thus produced. However, in the present study it is demonstrated that if a muscarine receptor antagonist (atropine or biperiden) is *combined* with an α-adrenergic agonist/α-adrenergic agonist precursor (clonidine or L-α-methyl-dopa), a marked locomotor stimulation can be achieved, although either agent given alone is ineffective. Adding an NMDA antagonist (MK-801, ketamine or SDZ EAA494) to the combination biperiden+clonidine resulted in further potentiation of the locomotor stimulatory effects.

Key words: Locomotion – Parkinson's disease, model of – Atropine – Clonidine – NMDA antagonist

Introduction

Traditionally, dopamine has been regarded as playing a crucial role in the initiation and execution of locomotion. However, in a series of studies we have demonstrated that, in at least one animal model of Parkinson's disease, locomotion can be restored by manipulating other transmitter systems than the dopaminergic. In our model, mice are depleted of their monoaminergic stores by reserpine, injected in a dose of 10 mg/kg 18 h before the acute experiment, and the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine (α-MT), injected in a dose of 250 mg/kg 60 min before the acute experiment. In previous experiments, we have found that the akinesia thus produced can be reversed by manipulating central glutamatergic, adrenergic and cholinergic systems (Carlsson and Carlsson 1989a, b; Carlsson and Svensson 1990a, b).

Briefly, what we have found is that the suppression of glutamatergic neurotransmission by administering high doses of noncompetitive (MK-801 = dizocilpine, phencyclidine or ketamine) or competitive (SDZ EAA494 = D-CPPene) NMDA antagonists causes locomotor stimulation in monoamine-depleted mice. Furthermore, we have found that administration of a low dose of an NMDA antagonist discloses the locomotor stimulatory potential of the α-adrenoceptor agonist clonidine, the muscarine receptor antagonist atropine or the dopamine receptor agonist apomorphine, the latter given in a subthreshold dose.

In contrast to apomorphine and other dopaminergic agents, an α-adrenergic agonist or a muscarinic antagonist given alone cannot restore locomotion in monoamine-depleted mice, even if given in large doses. However, in a preliminary study we have observed that *simultaneous* manipulations of central adrenergic and cholinergic systems have profound effects on locomotion; a high dose of the α-adrenergic agonist clonidine combined with a high dose of the muscarine receptor agonist atropine caused a pronounced stimulation of locomotion, and this response was counteracted by the acetylcholine esterase inhibitor physostigmine as well as by the selective α_2-adrenoceptor blocker idazoxan, belonging to the imidazole family (Carlsson and Carlsson 1989c).

The aims of the present investigation were (1) to establish the involvement of central muscarinic receptors and α_2-adrenoceptors in the locomotor response produced by the combined atropine and clonidine treatment (a) by observing the effects of an alternative adrenergic (L-α-methyl-dopa) and muscarinic agent (biperiden) and (b) by testing whether the nonimidazole α_2-adrenoceptor-selective antagonist yohimbine and the atypical neuroleptic clozapine (which is also an α_2-adrenoceptor antagonist) are able to block the locomotor response;

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(2) to elucidate whether the addition of an NMDA antagonist to treatment with an adrenergic agonist in combination with a low dose of a muscarinic antagonist might have a potentiating effect on locomotion in monoamine-depleted mice.

Methods

Animals. Male albino mice of the NMRI strain (20–30 g) were purchased from ALAB, Sollentuna.

Drugs. Reserpine (Ciba-Geigy, Basel), yohimbine HCl (Sigma, St. Louis), prazosin HCl (Pfizer, New York) and clozapine (Wander, Berne) were dissolved in a few drops of glacial acetic acid and 5.5% glucose solution. Idazoxan (Reckitt and Colman, Hull) was dissolved in distilled water. Haloperidol (Leo, Helsingborg) was dissolved in a few drops of glacial acetic acid and physiological saline. Biperiden lactate (Akineton, Knoll/Meda, Göteborg) was dissolved in distilled water containing 14 mg per ml of sodium lactate. α-Methyl-para-tyrosine methylester HCl (α-MT; Sigma), clonidine HCl (Boehringer Ingelheim, FRG), L-α-methyl-dopa (Sigma), atropine sulphate (Aldrich, Steinheim), MK-801 ([(+)-5-methyl-10,11-di-hydroxy-5*H*-dibenzo(*a*,*d*)-cyclohepten-5,10-imine]hydrogen maleate; dizocilpine; Research Biochemical Inc. Naticle). SDZ EAA 494 (D-CPPene; [3-(2-carboxypiperazine-4-yl)-1-propenyl-1-phosphonic acid]; courtesy of Dr. PL Herrling at Sandoz Research Institute, Berne, Switzerland), HCl (Sigma), raclopride (Astra, Södertälje) and SCH 23390 [(R)-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol; Schering, Berlin] were dissolved in physiological saline. All drugs were dissolved in an ultrasonic bath and injected i.p. in a volume of 10 ml/kg, exept for atropine, raclopride and SCH 23390, which were given s.c. and reserpine, which was given in a volume of 20 ml/kg. Appropriate vehicle treatment was always given so that all mice in any one experiment received the same injection volume. Doses are given in the figure legends and refer to the salts of the drugs.

Procedure. In the experiments in which an NMDA antagonist was included, the model for measuring motor activity consisted of a circular track, 5 cm wide and 1 m in circumference, the inner and outer walls being transparent plastic cylinders, 15 and 25 cm high, respectively. The number of turns (= meters) the animal covered in 30 min was registered by means of IR detectors. The reason for using circular tracks when quantifying motor activity in monoamine-depleted mice treated with NMDA antagonists is that these animals display only forward locomotion and are thus unable to change direction when reaching a corner. In the other experiments motor activity was measured by means of an "M/P 40 Fc Electronic Motility Meter" (Motron Products, Stockholm) with 40 photoconductive sensors (5 rows×8, centre-centre distance 40 mm). All animals in this study received reserpine (10 mg/kg i.p.) 18 h prior to the acute experiment. One hour after the animals had been injected with reserpine and throughout the experiment the ambient temperature was held at 28 °C. The behaviour and gross appearance of the animals were observed throughout the experiments.

Results

All mice used in the present study were depleted of monoaminergic stores by means of reserpine (10 mg/kg, 18 h) and α-MT (250 mg/kg) pretreatment with the aims of (1) obtaining an akinesia model mimicking Parkinson's disease and (2) enabling studies on postsynaptic interactions between cholinergic, adrenergic and glutamatergic systems.

Figure 1 illustrates the synergistic interaction between the muscarine receptor antagonist atropine (40 mg/kg) and the α-adrenergic agonist clonidine with regard to locomotor stimulation in monoamine-depleted mice:

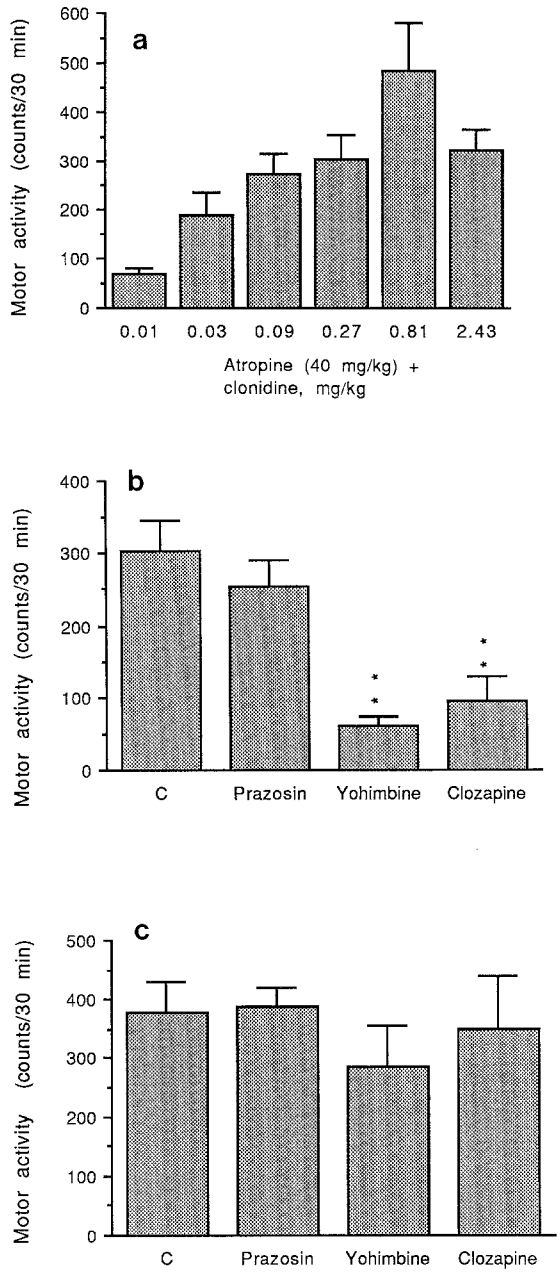

Fig. 1. a Effects of various doses of clonidine, combined with 40 mg/kg of atropine, on locomotion in monoamine-depleted mice. α-MT was administered 90 min, atropine 30 min and clonidine immediately before the locomotor activity registration started. Motor activity was registered during 30 min. Shown are the means ± SEM (n = 4). There was a significant correlation between clonidine dose in the dose range 0.01–0.81 mg/kg and motility counts ($r = 0.74$, $p < 0.001$). **b** Effects of different catecholamine receptor blockers on the locomotor stimulation induced by 40 mg/kg of atropine, combined with a low (0.09 mg/kg) dose of clonidine in monoamine-depleted mice. α-MT was administered 90 min, atropine 35 min and prazosin (1 mg/kg), yohimbine (6 mg/kg) and clozapine (20 mg/kg) 30 min prior to clonidine treatment. Motor activity was registered during 30 min, beginning 5 min after the clonidine injection. Shown are the means ± SEM (n = 6). **$p < 0.01$ vs. controls (C) (Mann-Whithney U-test). **c.** Lack of effect of different catecholamine receptor blockers on the locomotor stimulation induced by 40 mg/kg of atropine, combined with a high (2 mg/kg) dose of clonidine in monoamine-depleted mice. α-MT was administered 2 h, prazosin (1 mg/kg), yohimbine (6 mg/kg) and clozapine (20 mg/kg) 90 min, and atropine 60 min prior to clonidine treatment. Motor activity was registered during 30 min, beginning 15 min after the clonidine injection. Shown are the means ± SEM (n = 8)

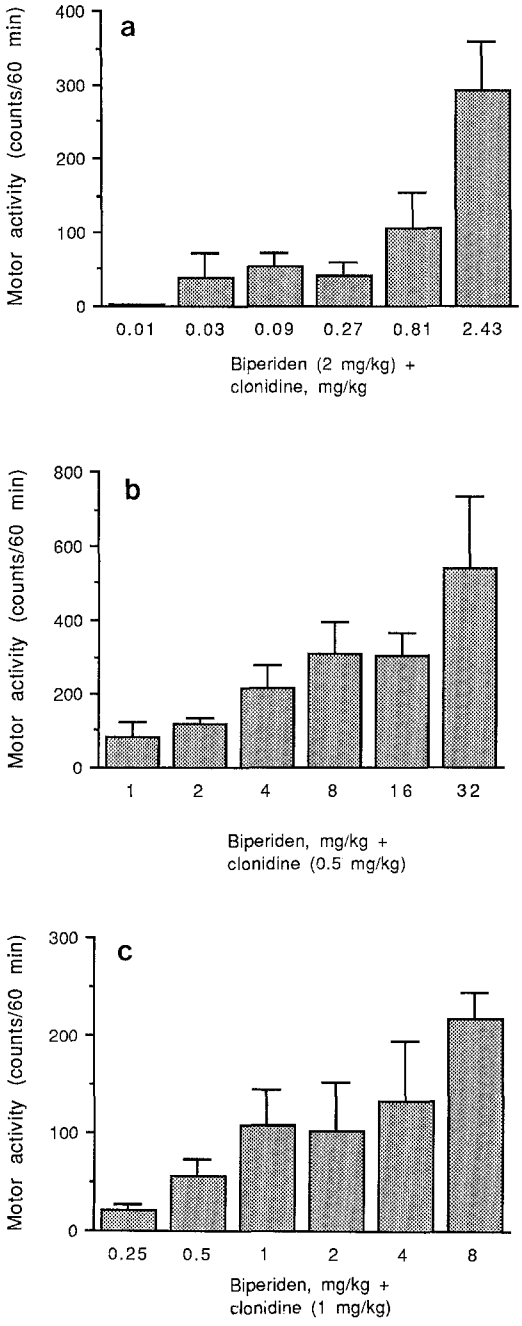

Fig. 2. a Effects of various doses of clonidine, combined with 2 mg/kg of biperiden, on locomotion in monoamine-depleted mice. α-MT was administered 2 h, biperiden 60 min and clonidine immediately before the locomotor activity registration started. Motor activity was registered during 60 min. Shown are the means ± SEM (n = 4). There was a significant correlation between clonidine dose and motility counts (r = 0.82, $p < 0.001$). **b** Effects of various doses (1 – 32 mg/kg) of biperiden, combined with 0.5 mg/kg of clonidine, on locomotion in monoamine-depleted mice. α-MT was administered 2 h, biperiden 60 min and clonidine immediately before the locomotor activity registration started. Motor activity was registered during 60 min. Shown are the means ± SEM (n = 4). There was a significant correlation between biperiden dose and motility counts (r = 0.63, $p < 0.001$). **c** Effects of various doses (0.25 – 8 mg/kg) of biperiden, combined with 1 mg/kg of clonidine, on locomotion in monoamine-depleted mice. α-MT was administered 2 h, biperiden 60 min and clonidine immediately before the locomotor activity registration started. Motor activity was registered during 60 min. Shown are the means ± SEM (n = 4). There was a significant correlation between biperiden dose and motility counts (r = 0.64, $p < 0.001$)

even with doses as low as 0.03 and 0.09 mg/kg of clonidine a pronounced behavioural stimulation is obtained. The locomotor stimulatory effect produced by treatment with 0.09 mg/kg of clonidine in combination with atropine was blocked by pretreatment with the selective α_2-adrenoceptor antagonist yohimbine and by the atypical neuroleptic clozapine, but not by the selective α_1-adrenoceptor antagonist prazosin. None of these agents was able to block the response induced by a high dose (2 mg/kg) of clonidine combined with atropine.

Untreated mice with intact monoamine stores normally accumulate up to 200 counts during the first 30 min and up to 300 counts during the first 60 min.

In order to confirm the involvement of central cholinergic systems in the observed response, dose-response studies were conducted with another muscarine receptor antagonist, i.e. biperiden, which is also clinically more interesting than atropine. Dose-related locomotor responses were obtained when increasing doses of biperiden were combined with a fixed dose of clonidine and vice versa (Fig. 2). Note that the scales differ in the various figures.

To establish the involvement of central adrenergic systems in the locomotor responses produced when a muscarinic antagonist is combined with clonidine, the non imidazole adrenergic agonist precursor L-α-methyldopa was given in various doses in combination with 40 mg/kg of atropine. In this case too, a clear-cut stimulation of locomotion was produced. The α_2-adrenoceptor blockers idazoxan and yohimbine antagonized the response, although in the case of yohimbine statistical significance was not reached. The α_1-adrenoceptor blocker prazosin, the preferential dopamine D_2 receptor blockers haloperidol and raclopride, and the dopamine D_1 blocker SCH 23390 all failed to attenuate the locomotor response (Fig. 3). Neither L-α-methyl-dopa nor clonidine, when given alone, can reverse the reserpine-induced akinesia (not shown).

Finally, the effects on locomotion produced when a glutamate antagonist was added to the combined biperiden + clonidine treatment were investigated. It was found that combining a low dose of the noncompetitive NMDA antagonist MK-801 or the competitive NMDA antagonist D-CPPene with biperiden + clonidine yielded a locomotor response that was significantly greater than if only two of the drugs were combined. When the noncompetitive NMDA antagonist ketamine was used, the locomotor response was significantly greater in the group receiving all three agents than in the groups receiving only biperiden + ketamine or ketamine + clonidine; it tended to be greater than the response in the group receiving the biperiden + clonidine combination, but this difference was not statistically significant (Fig. 4).

Discussion

As previously described (Carlsson and Carlsson 1989c), monoamine-depleted mice receiving a muscarinic antagonist in combination with an α-adrenergic agonist display a varied pattern of locomotion, including both horizontal

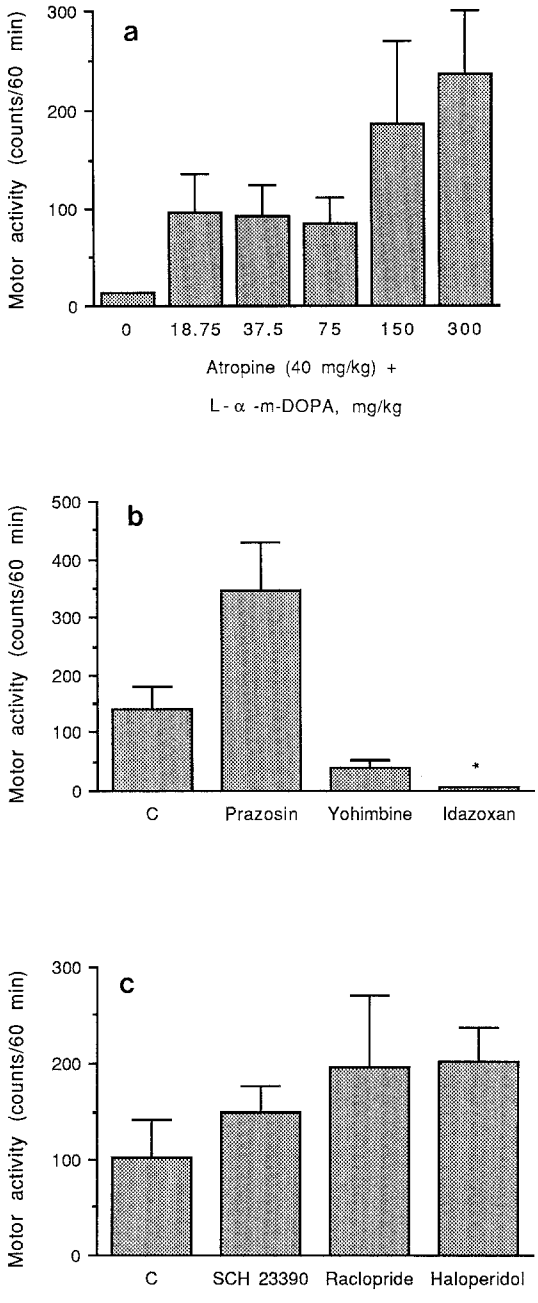

Fig. 3. **a** Effects of various doses of L-α-m-dopa, combined with 40 mg/kg of atropine, on locomotion in monoamine-depleted mice. α-MT was administered 2 h, and L-α-m-dopa and atropine 60 min before the locomotor activity registration started. Motor activity was registered during 60 min. Shown are the means ± SEM (n = 4). There was a significant correlation between L-α-m-dopa dose and motility counts ($r = 0.53$, $p < 0.02$). **b** Effects of α-adrenergic blockers on the locomotor stimulation induced by L-α-m-dopa (300 mg/kg) and atropine (40 mg/kg) treatment. α-MT was administered 2 h, and L-α-m-dopa, atropine, prazosin (1 mg/kg), yohimbine (6 mg/kg) and idazoxan (10 mg/kg) 60 min before the locomotor activity registration started. Motor activity was registered during 60 min. Shown are the means ± SEM (n = 5–6). *$p < 0.05$ vs. controls (C) (Mann-Whitney U-test). **c** Effects of dopamine receptor blockers on the locomotor stimulation induced by L-α-m-dopa (150 mg/kg) and atropine (40 mg/kg) treatment. α-MT was administered 2 h, and L-α-m-dopa, atropine, raclopride (20 mg/kg) and haloperidol (1 mg/kg) 60 min before the locomotor activity registration started. SCH 23390 (0.3 mg/kg) was injected 30 min as well as immediately before locomotor activity registration started. Motor activity was registered during 60 min. Shown are the means ± SEM (n = 6)

and vertical (jumping) movements; thus, when reaching a wall or a corner they are able to change direction. This is in contrast to monoamine-depleted mice receiving an NMDA antagonist; these animals display only forward locomotion and therefore get stuck in corners (Carlsson and Carlsson 1989a).

The present study reveals that, when combined with a high dose of atropine, very low doses (0.03 or 0.09 mg/kg) of clonidine can induce pronounced locomotor stimulation. When combined with MK-801, on the other hand, clonidine needs to be given in higher doses to reverse the reserpine-induced akinesia (Carlsson and Svensson 1990b). In addition, the locomotor response produced by 1 mg/kg of MK-801 combined with 2 mg/kg of clonidine was readily antagonized by both the selective α_2-adrenergic blockers idazoxan and yohimbine and the atypical neuroleptic clozapine (which also blocks α-adrenergic receptors; Carlsson 1978; Perry et al. 1983; Ramirez and Wang 1986). However, when such a high dose of clonidine was used in combination with atropine, idazoxan was found to prevent the locomotor response (Carlsson and Carlsson 1989c), whereas yohimbine and clozapine were ineffective, as evident from the present study. On the other hand, yohimbine and clozapine effectively antagonized the locomotor response induced by atropine combined with a *low* dose (0.09 mg/kg) of clonidine. The selective α_1-adrenergic blocker prazosin has been found consistently ineffective. Taken together, these results suggest that the population of α_2-adrenergic receptors involved in the locomotor stimulatory effect differs, depending on whether clonidine is combined with the NMDA antagonist MK-801 or with the muscarine receptor antagonist atropine.

Supporting a role for α-adrenergic receptors, as opposed to the so-called imidazole-preferring receptor (see Bylund 1988), in the locomotor response produced by the combined atropine and clonidine treatment, are (a) the observation that also the nonimidazole blockers yohimbine and clozapine were effective in antagonizing the response when a low dose of clonidine was used and (b) the observation that the nonimidazole adrenergic agonist precursor L-α-methyl-dopa, given in combination with atropine, was also highly effective in producing a locomotor stimulatory effect. Underlining the importance of α_2-adrenoceptors, rather than dopamine receptors, in this context, is the observation that the α_2-adrenoceptor blockers idazoxan and yohimbine antagonized the locomotor response, whereas the dopamine receptor blockers haloperidol, raclopride and SCH 23390 were unable to attenuate the response (cf. Andén and Strömbom 1975; Andén 1979).

The experiments undertaken with the anticholinergic agent biperiden had two purposes. The first was to establish the involvement of central muscarinic receptors in the observed locomotor response. In conjunction with the previously reported antagonizing effect of the acetylcholinesterase inhibitor physostigmine, the finding that biperiden, similarly to atropine, produced a clear-cut locomotor stimulation when combined with clonidine, firmly supports a role for central muscarinic receptors. The second was to prepare the ground for possible future

clinical trials in Parkinsonian patients. To that end it was important to demonstrate the efficacy of a clinically relevant anticholinergic agent, such as biperiden. The various dose-response studies were also conducted with an eye to possible clinical studies. From these mouse experiments it appears that the doses of both the anticholinergic and the adrenergic agent can be kept at reasonably low levels without losing too much in effect; furthermore, it must be kept in mind in this context that rodents usually need considerably larger doses than humans for the same level of effect.

The addition of an NMDA antagonist to the combined biperiden+clonidine treatment was also carried out with possible clinical trials in Parkinsonian patients in mind. Conceivably, if our mouse experiments have any relevance for man, addition of a low dose of a glutamatergic antagonist might be beneficial, should a muscarinic antagonist in combination with an adrenergic agonist not suffice.

The present and previous studies (see Carlsson and Carlsson 1990) have opened up new perspectives on the role played by different neurotransmitter systems for motor functions. Hopefully this new knowledge, based on findings in experimental animals, will aid in finding novel strategies for treating movement disorders like Parkinson's disease. Conceivable, in the future, if our animal data can be extrapolated to man, the pharmacological treatment of Parkinson's disease could be optimized by titrating a tailor-made "cocktail" containing varying proportions of a dopaminergic agonist, a glutamatergic antagonist, a muscarinic antagonist and/or an adrenergic agonist, taking advantage of synergistic effects with respect to locomotion to allow low dosage and thus, hopefully, to minimize side-effects. Needless to say, there is a considerable risk that side-effects produced by muscarinic and glutamatergic antagonists, such as confusion and memory impairment, cannot be avoided even if doses are kept to a minimum. If this is the case, the hope for the future may be to find pharmacological tools with selectivity for receptor subtypes to make it possible to differentiale between effects on motor and cognitive functions.

Acknowledgements. The present study was supported by grants from MFR (155 and 9067), Stiftelsen för Gamla Tjänarinnor, Hans och Loo Ostermans Fond, Torsten och Ragnar Söderbergs Stiftelser, Stiftelsen Sigurd och Elsa Goljes Minne, Socialstyrelsens Fonder, Stiftelsen Handlanden Hjalmar Svenssons Forskningsfond, Åhlénstiftelsen, Gun och Bertil Stohnes Stiftelse and Syskonen Svenssons Fond. We are indebted to Malin Lundgren for skillful technical assistance. We also wish to thank Dr. P.L. Herrling at Sandoz Research Institute, Berne, for generously supplying SDZ EAA 494.

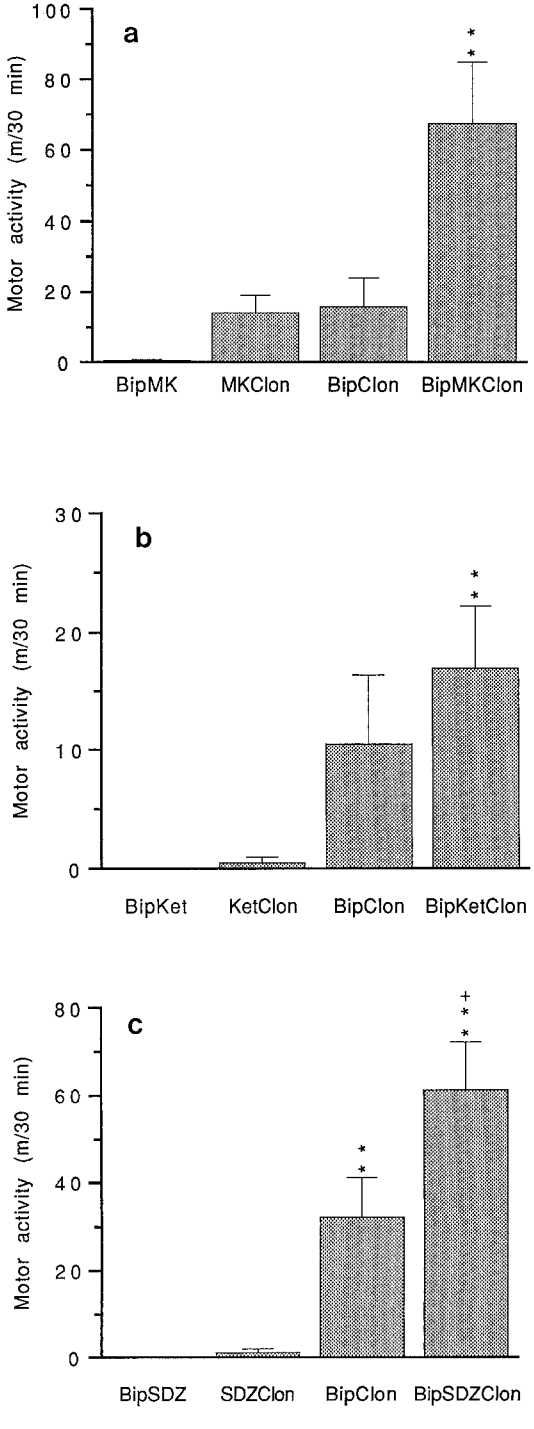

Fig. 4. **a** Effects of biperiden and clonidine in combination with MK-801 on motor activity in monoamine-depleted mice. α-MT was administered 2 h, biperiden (1 mg/kg) and MK-801 (0.2 mg/kg) 60 min, and clonidine (0.5 mg/kg) immediately before the locomotor activity registration started. Motor activity was registered during 30 min. Shown are the means±SEM (n = 6). **$p < 0.02$ vs. the other groups (Mann-Whitney U-test). **b** Effects of biperiden and clonidine in combination with ketamine on motor activity in monoamine-depleted mice. α-MT was administered 2 h, biperiden (1 mg/kg) and ketamine (12.5 mg/kg) 60 min, and clonidine (0.5 mg/kg) immediately before the locomotor activity registration started. Motor activity was registered during 30 min. Shown are the means±SEM (n = 6) **$p < 0.01$ vs. the group receiving biperiden in combination with ketamine and the group receiving ketamine in combination with clonidine (Mann-Whitney U-test). **c** Effects of biperiden and clonidine in combination with SDZ EAA 494 on motor activity in monoamine-depleted mice. α-MT was administered 2 h, biperiden (1 mg/kg) and SDZ EAA 494 (1 mg/kg) 60 min, and clonidine (0.5 mg/kg) immediately before the locomotor activity registration started. Motor activity was registered during 30 min. Shown are the means±SEM (n = 6). **$p < 0.01$ vs. the groups receiving biperiden in combination with SDZ EAA 494 and SDZ EAA 494 in combination with clonidine; *$p < 0.05$ vs. the group receiving biperiden in combination with clonidine (Mann-Whitney U-test)

References

Andén N-E (1979) Selective stimulation of central α-autoreceptors following treatment with α-methyldopa and FLA 136. Naunyn-Schmiedeberg's Arch Pharmacol 306:263–266

Andén N-E, Strömbom U (1975) Stimulation of central adrenergic alpha-receptors by L-dopa, α-methyldopa and clonidine. In: Davies DS, Reid JL (eds) Central action of drugs in blood pressure regulation. Pitman Medical, Tunbridge Wells, pp 225–236

Bylund DB (1988) Subtypes of α_2-adrenoceptors: pharmacological and molecular biological evidence converge. Trends Pharmacol Sci 9:356–361

Carlsson A (1978) Antipsychotic drugs, neurotransmitters, and schizophrenia. Am J Psychiatry 135:164–173

Carlsson M, Carlsson A (1989a) The NMDA antagonist MK-801 causes marked locomotor stimulation in monoamine-depleted mice. J Neural Transm 75:221–226

Carlsson M, Carlsson A (1989b) Dramatic synergism between MK-801 and clonidine with respect to locomotor stimulatory effect in monoamine-depleted mice. J Neural Transm 77:65–71

Carlsson M, Carlsson A (1989c) Marked locomotor stimulation in monoamine-depleted mice following treatment with atropine in combination with clonidine. J Neural Transm 1:317–322 (P-D Sect)

Carlsson M, Carlsson A (1990) Interactions between glutamatergic and monoaminergic systems within the basal ganglia – implications for schizophrenia and Parkinson's disease. Trends Neurosci 13:272–276

Carlsson M, Svensson A (1990a) Interfering with glutamatergic neurotransmission by means of NMDA antagonist administration discloses the locomotor stimulatory potential of other transmitter systems. Pharmacol Biochem Behav 36:45–50

Carlsson M, Svensson A (1990b) The non-competitive NMDA antagonists MK-801 and PCP, as well as the competitive NMDA antagonist SDZ EAA 494 (D-CPPene), interact synergistically with clonidine to promote locomotion in monoamine-depleted mice. Life Sci 47:1729–1736

Perry BD, Simon PR, U'Prichard DC (1983) Interactions of neuroleptic compounds at α_2-adrenergic receptor affinity states in bovine caudate nucleus. Eur J Pharmacol 95:315–318

Ramirez OA, Wang RY (1986) Locus coeruleus norepinephrine-containing neurons: effects produced by acute and subchronic treatment with antipsychotic drugs and amphetamine. Brain Res 362:165–170