2 Schaefer, K.E., Hastings, B.J., Carey, C.R., and Nichols, Jr, G., Respiratory acclimatization to carbon dioxide. J. appl. Physiol. *18* (1963) 1071–1078.
3 Guillerm, R., and Radzisewski, E., Effects on many of 30-day exposure to $PICO_2 = 14$ torr (2%): application to exposure limits. Undersea Biomed. Res., Suppl. *6* (1979) S91–S114.
4 Schaefer, K.E., Respiration and acid-base balance during prolonged exposure to 3% CO_2. Pflügers Arch. *25* (1949) 689–694.
5 Clark, J.M., Sinclair, R.D., and Welch, B.E., Rate of acclimatization to chronic hypercapnia in man, in: Underwater Physiology, pp.399–408. Ed. C.J. Lambertsen. Academic Press, New York 1971.
6 Schaefer, K.E., Nichols, Jr., G., and Carey, C.R., Acid-base balance and blood using electrolytes of man during acclimatization to carbon dioxide. J. appl. Physiol. *19* (1964) 48–58.
7 Polak, A., Haynie, G.D., Hayes, R.M., and Schwartz, W.B., Effect of chronic hypercapnia on electrolyte and acid-base equilibrium. I. Adaptation. J. clin. Invest. *40* (1961) 1223–1237.
8 Schwartz, W.B., Brackett, Jr, N.C., and Cohen, J.J., The response of extracellular hydrogen ion concentration to graded degrees of chronic hypercapnia: the physiological limits of the defense of pH. J. clin. Invest. *44* (1965) 291–301.
9 van Ypersele de Strihou, Brasseur, C.E.L., and DeConincok, J., The carbon dioxide response curve for chronic hypercapnia in man. N. Engl. J. Med. *275* (1966) 117–125.
10 Schaefer, K.E., Nichols, G., and Carey, C.R., Calcium phosphorus metabolism in man during acclimatization to carbon dioxide. J. appl. Physiol. *18* (1963) 1079–1084.
11 Messier, A.A., Heyder, E., Braithwaite, W.R., McCluggage, C., Peck, A., and Schaefer, K.E., Calcium, magnesium, and phosphorous metabolism and parathyroid-calcitonin function during prolonged exposure to elevated CO_2 concentrations on submarines. Undersea Biomed. Res., Suppl. *6* (1979) S57–S70.
12 Davies, D.M., Smith, D.J., Leitch, D.R., Morris, J.E.W., and Gray, S.P., The effects on man of continuous exposure to 0.5% carbon dioxide. Inst. nav. Med. Rep. 1976. No.22/76. Alverstoke, England.
13 Davies, D.M., Sixty days in a submarine: the pathophysiological and metabolic cost. J.R. Coll. Physicians *7* (1973) 132–144.
14 Poyart, C.F., Bursaux, E., and Freminet, A., The bone CO_2 compartment: evidence for a bicarbonate pool. Resp. Physiol. *25* (1975) 89–99.
15 Poyart, C.F., Freminet, A., and Bursaux., E. The exchange of bone CO_2 in vivo. Resp. Physiol. *25* (1975) 101–107.
16 Davies, D.M., and Morris, J.E.W., Carbon dioxide and vitamin D effects on calcium metabolism in nuclear submarines. Undersea Biomed. Res., Suppl. *6* (1979) S71–S80.
17 Reichart, E., Caludon, F. and Sabliere, S., CO_2 storage in various organs during chronic experimental hypercapnia. Bull. Eur. Physiopath. Resp. *12* (1976) 19–32.
18 Brown, Jr, E.B., and Michel, C.C., Whole body CO_2 exchange. Proc. XXIV Int. Congr. physiol. Sci. *6* (1968) S185–S186.
19 Schaefer, K.E., Pasquale, S., Messier, A.A., and Shea, M., Phasic changes in bone CO_2 fractions, calcium, and phosphorus during chronic hypercapnia. J. appl. Physiol; Resp. envir. Exercise Physiol. *48* (1980) 802–811.
20 Pasquale, S., Messier, A.A., Shea, M.L., and Schaefer, K.E., Bone CO_2 titration curves in acute hypercapnia. J. appl. Physiol, Resp. envir. Exercise Physiol. *48* (1980) 197–200.
21 Tenney, S.M., and Naitove, A., Effects of alveolar oxygen and carbon dioxide on gastric secretion. Fedn Proc. *19* (1960) 190.
22 Blakemore, W.S., and Wolfson, S.K., Respiratory insufficiency as a factor in postoperative gastrointestinal bleeding. J. thorac. cardiovasc. Surg. *44* (1962) 494–505.
23 Phil, B.G., Pohl, A.L., Dickens, R.A., and Glotzer, C.J., Effect of chronic hypercapnia on gastric secretion in the dog. Ann. Surg. *165* (1967) 254–265.
24 Schaefer, K.E., Carey, C.R., Dougherty, J.H., Messier, A.A., and Morgan C., Effect of intermittent exposure to 3% CO_2 on respiration, acid-base balance and calcium metabolism. Undersea Biomed. Res., Suppl. *6* (1979) S115–S134.
25 Schaefer, K.E., Niemoeller, H., Messier, A.A., Heyder, E., and Spencer, J., Chronic CO_2 toxicity: Species difference in physiological and histopathological effects. NAVSUB-MEDRSCHLAB Report 1971, No.656.
26 Schaefer, K.E., Hasson, M., and Niemoeller, H., Effect of prolonged exposure to 15% CO_2 on calcium and phosphorus metabolism. Proc. Soc. exp. Biol. Med. *107* (1961) 355–359.
27 Schaefer, K.E., Avery, M.E., and Bensch, K., Time course of changes in surface tension and morphology of alveolar epithelial cells in CO_2 induced hyaline membrane disease. J. clin. Invest. *43* (1964) 2080–2093.
28 Schaefer, K.E., Pasquale, S.M., Messier, A.A., and Niemoeller, H., CO_2 induced kidney calcification. Undersea Biomed. Res., Suppl. *6* (1979) S143–S153.
29 Cohn, D.V., Bowden, R., and Eller, G.J., The effect of parthyroid hormone in vivo on the accumulation of calcium and phosphate by kidney and on kidney mitochondrial function. J. biol. Chem. *242* (1967) 1253–1255.
30 Mahgoub, A., and Stern, P., Carbon dioxide and the effect of parathyroid hormone on bone in vitro. Am. J. Physiol. *226* (1974) 1272–1275.
31 Crumb, Ch.K., Martinez-Muldonado, M., Eknoyan, G., and Suki, X., Effect of volume expansion, purified parathyroid extract and calcium on renal bicarbonate absorption in the dog. J. clin. Invest. *54* (1974) 1287–1294.
32 Schaefer, K.E., Douglas, W.H.J., Messier, A.A., Shea, M.L., and Gohman, D.A., Effects of prolonged exposure to 0.5% CO_2 on kidney calcification and ultra structure of lungs. Undersea Biomed. Res., Suppl. *6* (1979) S155–S161.
33 Shea, M.L., Messier, A.A., and Bondi, K.R., Effect of prolonged exposure to low levels of CO_2 concentration on kidney calcium and lung ultra structure. Fedn Proc. *40* (1981) 675.
34 Douglas, W.H.J., Schaefer, K.E., Messier, A.A., and Pasquale, S.M., Proliferation of pneumocyte II cells in prolonged exposure to 1% CO_2. Undersea Biomed. Res., Suppl. *6* (1979) S135–S142.

Modulation of dopaminergic transmission by alpha-noradrenergic agonists and antagonists: Evidence for antidopaminergic properties of some alpha antagonists[1]

Peter C. Waldmeier[2], Rainer Ortmann and Serge Bischoff

Research Department, Pharmaceuticals Division, Ciba-Geigy Ltd, CH-4002 Basle (Switzerland), 27 May 1982

Summary. The effects on dopamine (DA) metabolism, on ^{3}H-spiperone binding and on amphetamine-induced stereotypies of a variety of drugs with different actions on $alpha_1$- and $alpha_2$-noradrenergic (NA) receptors have been investigated.

The preferential $alpha_2$-antagonists yohimbine, rauwolscine, piperoxane and esproquin as well as the preferential $alpha_1$-antagonists corynanthine and WB4101 increased homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) in the rat striatum, mesolimbic area, and cortex. Prazosine and clonidine tended to

reduce HVA and DOPAC. The preferential alpha$_2$-antagonists, tolazoline and RX-781094A, had no measurable effects on DA metabolism even at high doses.
Those compounds which in comparable doses increased DA metabolism inhibited ^{3}H-spiperone binding in the hippocampus. The effects in the striatum and cortex were smaller and did not show a relation to those in hippocampus or on DA metabolism. Only the yohimbine alkaloids antagonized amphetamine-induced stereotypies.
The results suggest that the effects on DA metabolism at least of yohimbine, rauwolscine, and corynanthine are related to their intrinsic antidopaminergic properties. The same might be true, although with a lesser degree of certainty, for piperoxane, esproquin, and WB4101.
Since many of the tested compounds possessing alpha-antagonistic properties interacted with the DA system, a close molecular relationship between alpha-noradrenergic and DA receptors might be anticipated. The preference of these compounds for the hippocampal subtype of DA receptors might indicate a particular role of the latter in the regulation of DA metabolism. On the other hand, the antagonism against haloperidol's enhancing effect on DA metabolism by clonidine suggests a modulatory NA influence on DA transmission. The observation that clonidine reduced the effects of yohimbine and piperoxane to a lesser degree than that of haloperidol, is in agreement with this notion.

Introduction

A considerable amount of evidence from pharmacological and biochemical investigations suggests the existence of interactions between noradrenergic and dopaminergic systems in the brain (for a detailed discussion, see Antelman and Caggiula, 1977). The principal facts can be summarized as follows. The preferential alpha$_2$-antagonist yohimbine increases dopamine (DA) turnover (Papeschi and Theiss, 1975; Anden et al., 1976; Anden and Grabowska, 1976), whereas clonidine in doses preferentially acting on alpha$_2$-receptors (Rochette and Bralet, 1975; Anden et al., 1976; Anden and Grabowska, 1976; Stroembom, 1976) and the alpha$_1$-antagonist prazosine (Alander et al., 1980) reduce DA turnover in DA-rich areas of the rat brain. Moreover, the effects of yohimbine on DA turnover is antagonized by a combination of clonidine and phenoxybenzamine (Anden and Grabowska, 1976).
Lesions of the noradrenergic ventral bundle reduces DA concentrations in the caudate nucleus and in other areas containing DA terminals, but increases it in the cell body regions (O'Donohue et al., 1979).
Alpha$_1$-agonists and alpha$_2$-antagonists were found to potentiate haloperidol catalepsy, whereas an antagonism was described for drugs with alpha$_1$-lytic or alpha$_2$-agonistic properties (Brown and Handley, 1979). This is controversial, however, since Al-Shabibi and Doggett (1978) reported an attenuation of haloperidol-induced catalepsy by both clonidine and yohimbine in similar doses.
Ipsiversive circling was induced in rats after unilateral locus coeruleus lesions; injection of phenoxybenzamine into the substantia nigra showed a similar effect, whereas noradrenaline (NA) injections caused contraversive rotation (Donaldson et al., 1979).
Scatton et al. (1980) have recently suggested that the effects of yohimbine on DA metabolism are due to intrinsic antidopaminergic properties of this compound rather than to its alpha-adrenoceptor blocking effects. The question, whether dopaminergic transmission is altered by drugs primarily affecting NA transmission, and if so, in which way, is of considerable importance with respect to the development of new drugs for the treatment of mental diseases. Therefore, we have studied the effects on DA metabolism of a number of compounds with different effects on alpha receptors, in order to examine whether they exhibit a consistent pattern of effects. Moreover, the effects on in vivo ^{3}H-spiperone binding and on amphetamine-induced stereotypies were assessed in an attempt to characterize possible direct antidopaminergic effects.

Materials and methods

Clonidine-HCl and 2-[2-(1,4-benzodioxanyl)]-2-imidazoline HCl (RX781094A) were kindly donated by Boehringer Sohn, Ingelheim, FRG, and Reckitt & Colman Ltd, Hull, England. Haloperidol was purchased from Cilag AG, Schaffhausen, Switzerland, and rauwolscine-HCl and corynanthine-HCl from Carl Roth, Karlsruhe, FRG. Esproquin-HCl, prazosine-HCl · H_2O and WB4101-HCl were synthesized in our Chemistry Department by Dres H. Schroeter, A. Storni, Th. Leutert and F. Ostermayer, respectively. Tolazoline-HCl is a product marketed by Ciba-Geigy (Priscol®).
For biochemical experiments, female Tif:RAIF(SPF) rats (Tierfarm Sisseln, Switzerland) weighing 160–200 g were used. For the spiperone binding experiments and amphetamine antagonism, male animals of the same strain and weight range were used.
Homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were isolated from the corpus striatum, the mesolimbic area (containing the nucleus accumbens and the tuberculum olfactorium as the major components; for the dissection procedure see Waldmeier and Maitre, 1976) and the neocortex on Sedaphex G 10 columns (Westerink and Korf, 1976) and quantified by HPLC with electrochemical detection (Waldmeier, 1980). A BAS system (Bioanalytical

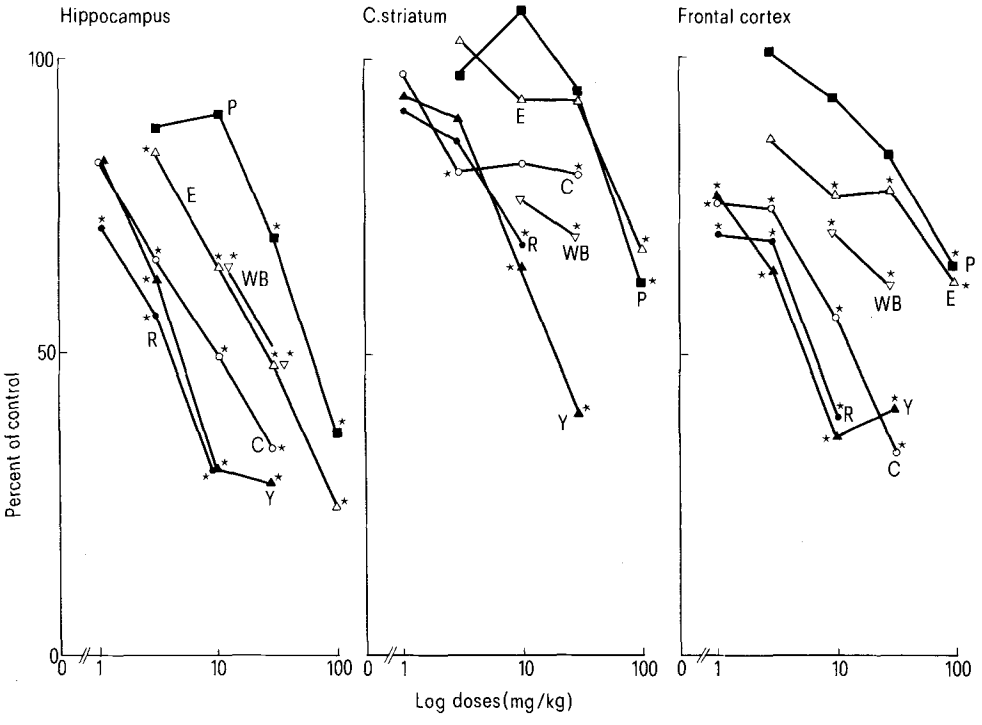

Figure 1. Effects of alpha-antagonists on in vivo ^{3}H-spiperone binding in rat hippocampus, striatum, and frontal cortex. Rats received the drugs i.p. 30 min before ^{3}H-spiperone and were decapitated 2 h thereafter. Data represent means in % of controls (n = 16 for controls. N = 8 for treated groups). Y, yohimbine; R, rauwolscine; C, corynanthine; E, esproquin; WB, WB4101; P, piperoxane.
Statistical calculations were done by means of Dunnett's test (* $p < 0.05$).

Systems, W. Lafayette, Ind., USA) with an LC4 controller, fitted with a $\frac{1}{8} \times 32$ inch glass-lined steel column filled with Zipax SAX anion exchanger (Dupont) was used. The mobile phase was a 4:1 mixture of 0.025 M acetate and citrate buffers of pH 5.0, pumped at 0.7 ml/min. The electrode potential was set at +0.8 V.

^{3}H-spiperone binding in vivo was determined as described by Bischoff et al. (1980). Rats were injected i.v. with 12 µCi ^{3}H-spiperone (25.6 Ci/mmol, New England Nuclear, Boston, Mass., USA) 30 min after the drugs to be tested, and decapitated 2 h thereafter. Non-specific spiperone binding was determined by pretreatment of the rats with 3 mg/kg i.p. cold spiperone 30 min before the injection of the labelled neuroleptic.

Antagonism of d-amphetamine-induced stereotypies was assessed in animals pretreated with the drugs 90 min before the injection of 7.5 mg/kg s.c. d-amphetamine. Licking and gnawing was evaluated 30, 40, 50 and 60 min after amphetamine administration. Scores were allotted for positive responses and the mean cumulative score calculated per group. Inhibition was calculated in percent of vehicle-injected controls and ED_{50}'s determined by log-plot regression analysis. For each dose 6–9 rats were used.

Results

1. *Effects of alpha-antagonists on DA metabolism in rat striatum, mesolimbic area, and cortex.* Yohimbine, WB4101 (table 1), esproquin, piperoxane, corynanthine, and rauwolscine (table 2) dose-dependently increased HVA and DOPAC in the striatum in a manner similar to neuroleptics. No such effect was observed with tolazoline up to a dose of 50 mg/kg i.p., and with RX781094A up to 30 mg/kg i.p. (table 1). Prazosine exhibited a tendency to lower the levels of the DA metabolites (table 2). However, its effect did not become statistically significant in this area at any of the doses tested.

The effects on mesolimbic and cortical DA metabolism were also investigated. In the mesolimbic area, the effects of some of the compounds on HVA levels

Table 1. Effects of some alpha-antagonists on striatal HVA and DOPAC concentrations

	Dose mg/kg	HVA ng/g	DOPAC ng/g
Controls	–	392 ± 38 (5)	671 ± 22 (5)
Yohimbine	1	762 ± 36* (5)	1056 ± 62* (5)
Yohimbine	2	1056 ± 54* (5)	1363 ± 52* (4)
Yohimbine	5	1449 ± 62* (5)	1715 ± 138* (5)
Controls	–	380 ± 30 (5)	770 ± 32 (5)
WB 4101	3	479 ± 28 (5)	796 ± 54 (5)
WB 4101	10	720 ± 97* (5)	1099 ± 92* (5)
WB 4101	30	1329 ± 25* (5)	1987 ± 67* (5)
Controls	–	446 ± 25 (4)	1420 ± 121 (5)
Tolazoline	25	486 ± 32 (5)	1446 ± 59 (5)
Tolazoline	50	479 ± 41 (5)	1478 ± 91 (5)
Controls	–	552 ± 43 (5)	1090 ± 15 (4)
RX 781094 A	1	596 ± 10 (4)	1025 ± 68 (5)
RX 781094 A	3	635 ± 26 (5)	1129 ± 42 (5)
RX 781094 A	10	637 ± 38 (5)	1078 ± 42 (5)
RX 781094 A	30	595 ± 46 (5)	953 ± 44 (5)

* $p < 0.01$ (Dunnett's test).
Rats were pretreated i.p. for 2 h. Data represent means ± SEM. The number of animals per group is indicated in brackets.

Table 2. Effects of some α-antagonists on striatal, mesolimbic and cortical HVA and DOPAC

		Corpus striatum HVA ng/g	DOPAC ng/g	Mesolimbic area HVA ng/g	DOPAC ng/g	Cortex HVA ng/g	DOPAC ng/g
Controls		442± 10 (5)	565± 28 (5)	184±17 (4)	548±52 (4)	13.7±1.3 (4)	11.4±2.4 (4)
Prazosine	1 mg/kg	393± 15 (5)	573± 26 (5)	134±11 (4)	377±30 (4)	-	10.3±1.5 (4)
	3 mg/kg	330± 13 (4)	522± 20 (4)	137± 7 (5)	477±24 (5)	10.8±1.1 (4)	9.0±1.8 (4)
	10 mg/kg	394± 33 (5)	543± 34 (5)	120±14* (4)	378±16 (4)	12.8±2.9 (3)	8.3±0.9 (4)
Esproquin	3 mg/kg	585± 55 (5)	681± 42 (5)	154±21 (5)	528±46 (5)	15.8±1.6 (4)	13.8±1.5 (4)
	10 mg/kg	1123± 72* (4)	1124± 76* (4)	246±16** (4)	630±83 (5)	30.1±6.4 (5)	15.1±3.6 (5)
	30 mg/kg	1393± 70* (5)	1366± 44* (5)	390±16* (5)	744±15** (5)	41.9±6.5* (5)	20.1±2.0 (5)
Piperoxane	3 mg/kg	471± 39 (5)	636± 56 (5)	174±13 (5)	518±19 (5)	10.7±1.5 (4)	9.4±1.9 (4)
	10 mg/kg	607± 54 (3)	735± 62 (5)	208±12 (5)	608±33 (5)	13.4±0.6 (3)	11.6±3.7 (3)
	30 mg/kg	1204± 62* (5)	1259± 60* (5)	356±11* (5)	662±17 (5)	37.4±9.1** (4)	18.1±6.3 (4)
Controls		564± 39 (5)	560± 38 (5)	261±27 (4)	624±30 (4)		12.9±1.3 (4)
Corynanthine	10 mg/kg	1024± 79* (5)	905± 29 (4)	372±37 (5)	765±79 (5)		15.1±2.4 (4)
	20 mg/kg	1724±129* (4)	2139±276* (4)	588±27* (5)	1041±18* (4)		25.4±2.5 (4)
Rauwolscine	1 mg/kg	816± 54 (5)	1032± 17 (4)	315±22 (5)	821±29 (5)		20.3±5.3 (4)
	2 mg/kg	1197± 46* (4)	1162± 64 (3)	440±23** (4)	799±34 (4)		21.0±2.4 (4)
	5 mg/kg	1447±112* (5)	1591±252* (5)	572±66* (5)	967±75* (5)		36.4±6.8* (4)

The animals were treated i.p. 2 h before decapitation. Data represent means ± SEM. The number of rats per group is indicated in brackets. * $p<0.01$; ** $p<0.05$; Dunnett's test.

were very similar to those in the striatum, whereas changes in DOPAC were less marked. In the cortex, dose-dependent increases of both HVA and DOPAC concentrations were also observed; as a consequence of the larger scatter, due to the small concentrations of the DA metabolites in this area, statistical significance was not always reached (table 2).

2. *Effects of alpha-antagonists on ^{3}H-spiperone binding in vivo in rat striatum, frontal cortex and hippocampus.* Yohimbine and rauwolscine were the most potent of the compounds tested with respect to the inhibition of ^{3}H-spiperone binding in all 3 areas investigated. However, in the striatum, the effect of both compounds was weaker than in the 2 other areas. Corynanthine was somewhat less potent than yohimbine and rauwolscine in hippocampus and frontal cortex, and showed remarkably little effect in the striatum: a plateau was reached at about 80% of controls between 3 and 30 mg/kg. Esproquin was 1/5–1/6 as potent than yohimbine or rauwolscine in the hippocampus; in the striatum the difference was greater (about 1/10), an appreciable effect being observed only with 100 mg/kg esproquin. A similar result was obtained in the frontal cortex. Piperoxane was less potent than esproquin in the hippocampus, but showed effects comparable to those of the latter agent in the other 2 areas (fig. 1). Prazosine was completely devoid of an inhibiting effect on ^{3}H-spiperone binding at 10 mg/kg i.p. in all 3 areas. The same was true for RX781094A at 10 mg/kg i.p.; at 50 mg/kg i.p., this compound reduced ^{3}H-spiperone binding in the hippocampus by 25%, but did not affect this parameter in the striatum and frontal cortex. Clonidine at 1 mg/kg i.p. was inactive in the hippocampus and frontal cortex but reduced ^{3}H-spiperone binding in the striatum by 15% at this dose (data not shown).

3. *Antagonism of amphetamine-induced stereotypies.* The effects of the alpha-antagonists on amphetamine-induced stereotypies are shown in table 3, together with summarized data on their effects on DA metabolism and ^{3}H-spiperone binding. Only the yohimbine alkaloids showed an appreciable inhibitory effect in the dose-range used; their ED_{50}'s were in the same order of magnitude as the ED_{50}'s for the inhibition of ^{3}H-spiperone binding in the hippocampus and the cortex and the ED_{200} for increasing striatal HVA. Piperoxane, esproquin, and WB4101, however, were completely inactive at 30 mg/kg i.p. Prazosine reduced amphetamine stereotypies by 30% at 10 mg/kg i.p.

4. *Absence of additivity of the effects of haloperidol and yohimbine.* Yohimbine (2 mg/kg i.p.) was administered alone or in combination with graded doses of haloperidol (0.05–0.3 mg/kg p.o.), in order to test whether the effects on striatal HVA concentrations were additive. In figure 2, the increases over controls produced by the individual drugs are compared with those of the combinations. Obviously, the effects of yohimbine and haloperidol were not additive.

5. *Effects of clonidine on the increases in DA metabolism elicited by haloperidol, yohimbine, and piperoxane.* Clonidine antagonized the increase in HVA and DOPAC concentrations in the rat striatum elicited by 0.3 mg/kg p.o. haloperidol. This effect was dose-dependent in the range of 0.01–0.3 mg/kg i.p. clonidine; it reached statistical significance at 0.03 mg/kg.

At the highest dose of clonidine, the effect of haloperidol on HVA and DOPAC levels was completely abolished. When given alone, clonidine caused minimal, but significant reductions of DOPAC at 0.1 and 0.3 mg/kg i.p. In other experiments not shown here, this effect of clonidine occasionally reached statistical significance also; in each case, it was marginal (fig. 3).
Clonidine antagonized the effect of 5 mg/kg i.p. yohimbine on striatal HVA (fig. 4). This inhibitory effect, too, was dose-dependent, but occurred only at 0.1 mg/kg i.p. of clonidine and above, and was much weaker in extent than that produced on the effect of haloperidol (cf. fig. 3).
Clonidine also significantly counteracted the HVA increase produced by 30 mg/kg i.p. piperoxane (fig. 4).

6. *Effects of WB 4101 and prazosine on the increases in DA metabolism in rat striatum, mesolimbic area and cortex, elicited by haloperidol.* Haloperidol at 0.3 mg/kg p.o. increased HVA and DOPAC levels in all 3 brain areas to a similar extent. WB4101, in doses which given alone did not change DA metabolism, was unable to counteract the effect of haloperidol in any of the areas. In contrast, prazosine, which showed a tendency to lower DA metabolism when given alone (in this experiment the effect on HVA in the striatum was significant; see also table 1), antagonized the effect of haloperidol at 10 mg/kg i.p. The effects on both HVA and DOPAC were significant in the cortex and mesolimbic area; in the striatum, only the effect on DOPAC was, due to the relatively large scatter in the HVA values (table 4).

Discussion

Of the drugs used in this study, yohimbine, rauwolscine, piperoxane, esproquin and tolazoline preferentially block alpha$_2$ (in general presynaptic) receptors (Borowski et al., 1976; Tanaka et al., 1978; Delini-Stula et al., 1979). RX781094A was reported to be very selective in this respect (Chapleo et al., 1981). Corynanthine predominantly blocks alpha$_1$ (in general postsynaptic) receptors (Weitzell et al., 1979) and prazosine does so very selectively (Cavero and Roach, 1980). WB4101 was initially regarded as a specific alpha$_1$ antagonist (U'Prichard and Snyder, 1979), but then it was found that the relative affinities of the drug for alpha$_1$- and alpha$_2$-receptors depends on the tissue selected (Hoffman and Lefkowitz, 1980). The preferential alpha$_2$-antagonists yohimbine, rauwolscine, esproquin, and piperoxane as well as the preferential alpha$_1$-antagonists corynanthine and WB4101, increased DA metabolism in a manner similar to neuroleptic drugs. Those which were tested in the mesolimbic area and the cortex showed a similar activity in these areas. In contrast, the preferential alpha$_2$-antagonists, tolazoline and RX781094A, were devoid of such an effect up to very high doses (50 and 30 mg/kg i.p., respectively). The same was true for the preferential alpha$_1$-antagonist, prazosine, which tended to reduce DA metabolism, if anything, in agreement with the results of previous investigators (Alander et al., 1980; Scatton et al., 1980).
Prazosine (at 3 mg/kg i.p. and above; Scatton et al., 1980), tolazoline (at 25 mg/kg i.p. and above; P. Baumann, personal communication) and RX781094A (at 10 mg/kg p.o. and above; P. Baumann, personal communication) significantly increased brain 3-methoxy-4-hydroxyphenylglycol sulphate; the latter 2 drugs antagonized the behavioral depression induced by clonidine already at lower doses (Delini-Stula et al., 1979; Dettmar et al., 1981). This indicates that these drugs possess in fact central effects at the

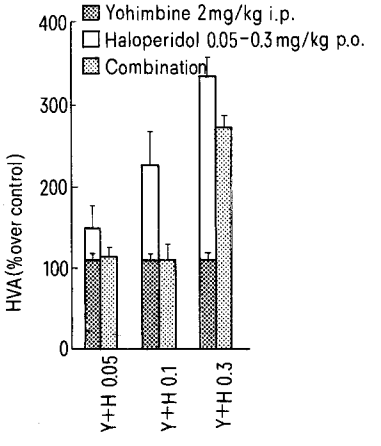

Figure 2. Absence of additivity of the effects of haloperidol and yohimbine on striatal HVA. Yohimbine (Y) was administered 30 min before graded doses of haloperidol (H). 2 h after the latter, the animals were sacrificed. Columns represent the HVA increase over controls in percent of controls ±SEM (n=5). The absolute control value of HVA was 569±12 ng/g.

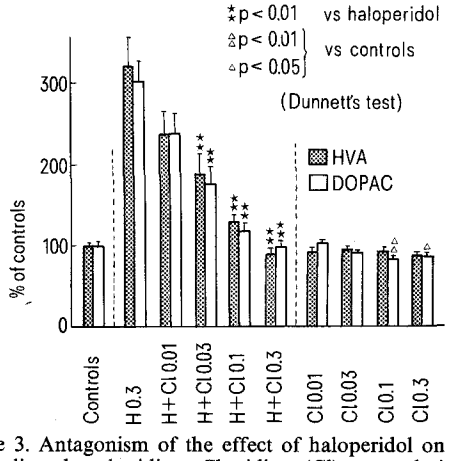

Figure 3. Antagonism of the effect of haloperidol on striatal DA metabolism by clonidine. Clonidine (Cl) was administered i.p. 30 min before haloperidol (H; 0.3 mg/kg p.o.). The animals were decapitated 2 h later. Data represent means ±SEM (n=5–10) in percent of controls. Absolute control levels were 364±10 ng/g (HVA) and 656±26 ng/g (DOPAC), Dunnett's test.

doses used in this study, and that their inability to increase DA metabolism cannot be ascribed to a poor penetration of the blood-brain barrier.

Thus, there is no consistent pattern emerging from our experiments: some, but not all preferential alpha$_2$-antagonists increase brain DA metabolism. On the other hand, such an effect was also observed with 2 preferential alpha$_1$-antagonists, but not with a third one. Therefore, our results do not seem to be readily compatible with the suggestion of Anden and Grabowska (1976) than DA metabolism is regulated directly or indirectly by postsynaptic alpha$_1$-receptors, and alpha$_2$-antagonists or -agonists increase or reduce, resp., the availability of NA at these by interfering with NA release. However, if some of the compounds possessed antidopaminergic properties of their own, this could account at least in part for the apparent discrepancy. The finding that the effects on DA metabolism of half-maximally active doses of yohimbine and haloperidol were less than additive is consistent with a mutual displacement of the 2 compounds at the same receptor, and, in fact, yohimbine has been suggested to exert its effects on DA metabolism by means of DA receptor blockade (Scatton et al., 1980). Further evidence for this, as well as for the other compounds increasing DA metabolism, comes from the in vivo ^{3}H-spiperone binding studies, and, at least for the yohimbine alkaloids, from the studies on the effect on amphetamine-induced stereotypies described under results.

All of the investigated compounds which had been found to increase DA metabolism, also interfered with ^{3}H-spiperone binding; conversely, those which did not increase DA metabolism did not affect ^{3}H-spiperone binding either. However, this effect was clearly more pronounced in the hippocampus than in the striatum or the frontal cortex.

Although an alpha-noradrenergic component of ^{3}H-spiperone binding has been suggested (Andorn and Maguire, 1980), the interaction of spiperone with alpha-receptors is rather weak. For instance, spiperone displaces ^{3}H-clonidine and ^{3}H-phentolamine in bovine retinal membranes with IC_{50} values of 1.4×10^{-6}M and 8×10^{-7}M, resp. ^{3}H-WB4101 is displaced from rat cortical membranes with an IC_{50} of 10^{-7}M, and the ED_{50} of spiperone to inhibit in vivo ^{3}H-WB4101 binding after pretreatment is above 3 mg/kg i.p. (H. Bittiger, personal communication). In comparison, spiperone displaces ^{3}H-spiperone from rat striatal membranes with an IC_{50} of 1.26×10^{-9}M (Leysen et al., 1978), and the ED_{50} for inhibition of in vivo ^{3}H-spiperone binding after pretreatment is 0.048 mg/kg i.p. (Bischoff et al., 1980). Therefore, it seems

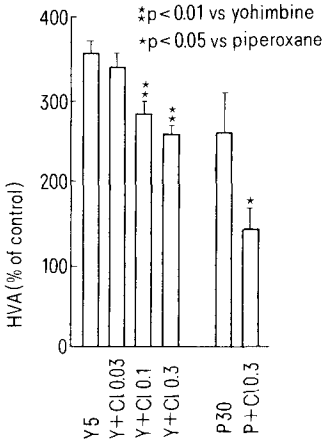

Figure 4. Antagonism of the effects of yohimbine and piperoxane on striatal HVA levels by clonidine. Clonidine (Cl) was administered i.p. 30 min before yohimbine (Y; 5 mg/kg i.p.) or piperoxane (P; 30 mg/kg i.p.) The animals were decapitated 2 h later. Data represent means ±SEM (n=5-10) in percent of controls. Absolute control levels of HVA were 395±21 ng/g (Dunnett's test).

Table 3. Comparison of the effects of alpha-antagonists on DA-metabolism, ^{3}H-spiperone binding and amphetamine stereotypies.

	ED_{200} HVA (striatum) mg/kg i.p.	ED_{50} hippocampus mg/kg i.p.	ED_{50} striatum mg/kg i.p.	ED_{50} frontal cortex mg/kg i.p.	ED_{50} antagonism of amphetamine stereotypies mg/kg i.p.
Yohimbine	1.1	4.6	17	6.25	3.2
Corynanthine	11	9.5	−20% at 30	12	10
Rauwolscine	1.8	3.8	−31% at 10	6.8	7
Esproquin	5.9	27	−32% at 100	−37% at 100	n.s. at 30
Piperoxane	17	58	−38% at 100	−34% at 100	n.s. at 30
WB4101	11	29	−28% at 30	−31% at 30	n.s. at 30
Prazosine		n.s. at 10	n.s. at 10	n.s. at 10	30% at 10
Clonidine		n.s. at 1	−15% at 1	n.s. at 1	
RX781094 A	n.s. at 30	n.s. at 10 −25% at 50	n.s. at 10 n.s. at 50	n.s. at 10 n.s. at 50	

To obtain a measure of the potencies of the drugs to alter DA metabolism, the dose doubling the baseline values of HVA in the striatum was determined by graphical interpolation from the dose-response curves given in tables 1 and 2. The ED_{50}'s with respect to inhibition of ^{3}H-spiperone binding were derived similarly from the data in figure 1. The effects on amphetamine-induced stereotypies (licking and gnawing) were evaluated 30, 40, 50 and 60 min after amphetamine administration (7.5 mg/kg s.c.) from dose-response curves (drugs given 90 min prior to amphetamine; n=6-9 per dose group). ED_{50}'s for the inhibition of amphetamine stereotypies were determined by log-plot regression analysis.

highly unlikely that the displacement of ^{3}H-spiperone in the hippocampus by the compounds in question is related to their effects on alpha receptors.

On the other hand, there is evidence that the sites labelled in vivo by ^{3}H-spiperone in the hippocampus are DA receptors. The main arguments are the following:

- there is an almost perfect correlation (Spearman rank coefficient $r=0.980$) between the effects of a large series of classical DA antagonists on in vivo ^{3}H-spiperone binding in the striatum and the hippocampus (Bischoff et al., 1981); also the sites in the latter become supersensitive after chronic haloperidol treatment (Bischoff, 1981).
- ^{3}H-spiperone does not seem to label 5-HT receptors in the hippocampus since 5 HT antagonists devoid of antidopaminergic properties are inactive (Bischoff et al., 1980), whereas DA antagonists without 5 HT antagonistic effects (e.g. sulpiride and tiapride) do inhibit ^{3}H-spiperone binding in this area (Bischoff et al., 1982).
- there is no correlation between the effects of a large number of drugs (including classical and atypical neuroleptics as well as 5 HT antagonists) on hippocampal and frontal cortical in vivo ^{3}H-spiperone binding. In the frontal cortex $^2/_3$ of the sites labelled by this ligand are 5-HT sites (Bischoff et al., 1980; Ortmann et al., in press).
- in contrast to what has been observed in vitro in the hippocampus and other limbic areas of the rat brain (Howlett et al., 1979), ^{3}H-spiperone does not seem to label a spirodecanone site in the hippocampus in vivo, since ^{3}H-spiperone binding in this area is almost completely inhibited by agents such as (+)-butaclamol and pimozide (Bischoff et al., 1981).

However, the DA receptors in the hippocampus seem to differ to some extent from those in the striatum. In particular, atypical neuroleptics like clozapine and thioridazine (Bischoff et al., 1980) and substituted benzamides (Bischoff et al., 1982) preferentially inhibited ^{3}H-spiperone binding in the hippocampus. It is, therefore, of particular interest to note that the alpha-antagonists showed a similar pattern of activity. Since parallelism of their effects on striatal DA metabolism was only found with their antagonism of ^{3}H-spiperone binding in the hippocampus and not in the striatum, it might be suspected that they block a subtype of DA receptor, occurring in the hippocampus in much greater proportion than in the striatum. One would have to assume, then, that such receptors also exist in the nigrostriatal and mesocortical systems and are involved in the regulation of DA metabolism.

In agreement with this, the amphetamine antagonism exerted by the yohimbine alkaloids occurred also in similar doses as the increases in DA metabolism and the inhibition of ^{3}H-spiperone binding in the hippocampus and frontal cortex; yet, piperoxane, esproquin, and WB4101 did not antagonize amphetamine sterotypies in comparable doses.

The results discussed so far suggest that the increase in DA metabolism produced by yohimbine, corynanthine and rauwolscine is related to antidopaminergic properties of these compounds. For piperoxane, esproquin, and WB4101, the ^{3}H-spiperone binding data also favor such a conclusion.

Some of our results support the idea of a noradrenergic modulation of dopaminergic neuronal activity, as suggested by Anden and Grabowska (1976). Thus, small doses of the preferential alpha$_2$-agonist, clonidine (0.03–0.3 mg/kg i.p.) strongly antagonized the increase in the acidic DA metabolites brought about by a maximally active dose of haloperidol (0.3 mg/kg). To counteract the corresponding effects of yohimbine (5 mg/kg i.p.) and piperoxane (30 mg/kg i.p.), higher doses of clonidine (0.1–0.3 mg/kg i.p.) were

Table 4. Effects on WB4101 and prazosine on the haloperidol-induced increase in DA metabolism.

	Corpus striatum		Mesolimbic area		Cortex	
	HVA ng/g	DOPAC ng/g	HVA ng/g	DOPAC ng/g	HVA ng/g	DOPAC ng/g
Controls	553± 10 (4)	1063+ 35 (5)	156± 6 (5)	454± 4 (4)	11.3±1.0 (5)	14.0±0.8 (5)
Haloperidol 0.3 mg/kg p.o.	1663±248 (5)	3054±341 (5)	478±43 (5)	1122± 46 (5)	37.3±3.3 (4)	32.0±2.8 (5)
WB4101 1 mg/kg i.p.	451± 31 (5)	982± 48 (5)	160±13 (15)	510± 24 (5)	11.8±1.5 (5)	15.0±1.8 (5)
WB4101 3 mg/kg i.p.	545± 45 (5)	943± 50 (5)	141±11 (14)	443± 46 (5)	14.8±1.0 (5)	12.5±1.0 (5)
Haloperidol + WB4101 1 mg/kg	1925±122 (5)	3519± 52 (4)	501±44 (5)	1154±116 (5)	38.8±2.5 (5)	29.3±1.0 (5)
Haloperidol + WB4101 3 mg/kg	1864±135 (5)	2944±188 (5)	589±10 (4)	1298± 48 (4)	50.0±7.8 (5)	34.5±6.0 (5)
Prazosine 3 mg/kg i.p.	425± 27 (4)	970± 39 (5)	165± 6 (5)	521± 6 (5)	11.5±1.0 (5)	14.5±1.3 (5)
Prazosine 10 mg/kg i.p.	395± 39[d] (5)	924± 37 (5)	141± 8 (5)	464± 36 (5)	12.8±0.5 (4)	11.0±0.5 (5)
Haloperidol + prazosine 3 mg/kg	1449± 47 (5)	2521±115 (5)	441± 6 (4)	1099± 43 (5)	34.0±1.8 (5)	28.5±5.5 (5)
Haloperidol + prazosine 10 mg/kg	994±251 (5)	1937±369[a](5)	238±24[b] (4)	759±114[a] (5)	17.8±2.5[b] (4)	16.3±2.8[b] (5)

Haloperidol was administered 2 h, WB4101 and prazosine 2.5 h before decapitation. Data represent means± SEM. The number of animals per group is indicated in brackets. [a] $p<0.01$, [b] $p<0.05$, vs haloperidol. [c] $p<0.01$, [d] $p<0.05$, vs controls. Dunnett's test.

needed, and the antagonism was weaker than in the case of haloperidol. Since there is no convincing evidence for a direct DA agonistic effect of clonidine, these results are compatible with the view that these reductions have been brought about by an action of this drug on alpha$_2$-receptors: the alpha$_2$-antagonistic properties of yohimbine and piperoxane partly prevented clonidine's decreasing effect on DA metabolism enhanced presumably by their antidopaminergic properties. In contrast, when haloperidol, which lacks alpha$_2$-antagonistic properties, was used to enhance DA metabolism, clonidine was very effective. It is noteworthy that the effect of clonidine on basal DA metabolism was much weaker than if it was enhanced by haloperidol. This latter result could also mean that alpha stimulation is particularly effective in reducing DA metabolism if the latter is in some way increased. Moreover, the alpha$_1$-antagonist, prazosine, was also found to antagonize the effect of haloperidol on DA metabolism. In turn, WB4101, in doses which per se did not increase DA metabolism, was unable to produce such an effect; it is possible that the alpha$_1$-antagonistic effects of this drug and those which cause the enhancement of DA metabolism appear in doses which are not sufficiently separated in vivo.

In conclusion, our results suggest that the increases in DA metabolism produced by yohimbine, rauwolscine and corynanthine are related to antidopaminergic properties of these compounds. The same can be said, although with lesser certainty, for piperoxane, esproquin, and WB4101. In fact, it is not particularly surprising that a number of compounds possessing alpha-antagonistic properties also block DA receptors; that there must be some degree of resemblance between these 2 types of receptors is also suggested by the fact that most neuroleptic agents possess quite substantial alpha-antagonistic properties. The close parallelism between the effect of the drugs tested in this study on DA metabolism and on the hippocampal subtype of the DA receptor might indicate that the latter plays a crucial role in the regulation of the former.

On the other hand, the ability of clonidine to counteract the increase in DA metabolism elicited by haloperidol documents the existence of a modulatory influence of NA neurons on dopaminergic transmission.

1 Part of this work has been presented at the 13th Meeting of the Union of Swiss Societies of Experimental Biology, Lausanne, March 26/27, 1981 (for abstract see Waldmeier and Bischoff, 1981).

2 Send reprints requests to Peter C. Waldmeier at the given address.

Alander, T., Anden, N.-E., and Grabowska, M., Metoclopramide and sulpiride as selective blocking agents of pre- and postsynaptic dopamine receptors. Naunyn-Schmiedeberg's Arch. Pharmac. *312* (1980) 145–150.

Al-Shabibi, U.M.H., and Doggett, N.S., On the central noradrenergic mechanism involved in haloperidol-induced catalepsy in the rat. J. Pharm. Pharmac. *30* (1978) 529–531.

Anden, N.-E., and Grabowska, M., Pharmacological evidence for a stimulation of dopamine neurons by noradrenaline neurons in the brain. Eur. J. Pharmac. *39* (1976) 275–282.

Anden, N.-E., Grabowska, M., and Stroembom, U., Different alpha-adrenoceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. Naunyn-Schmiedeberg's Arch. Pharmac. *292* (1976) 43–52.

Andorn, A.C., and Maguire, M.E., ^{3}H-Spiroperidol binding in rat striatum: Two high affinity sites of differing selectivities. J. Neurochem. *35* (1980) 1105–1113.

Antelman, S.M., and Caggiula, A.R., Norepinephrine-dopamine interactions and behaviour. Science *195* (1977) 646–653.

Bischoff, S., Bittiger, H., and Krauss, J., In vivo ^{3}H-spiperone binding to the rat hippocampal formation: involvement of dopamine receptors. Eur. J. Pharmac. *68* (1980) 305–315.

Bischoff, S., Increase in in vivo ^{3}H-spiperone binding in the rat hippocampal formation and striatum after repeated treatment with haloperidol. Experientia *37* (1981) 1008.

Bischoff, S., Bittiger, H., Delini-Stula, A., Ortmann, R., and Waldmeier, P., Further characterization of dopamine (DA) receptors in the rat hippocampal formation. 8th Meeting Int. Soc. Neurochem., Nottingham, U.K., September 7–11, 1981; abstr. p.39.

Bischoff, S., Bittiger, H., Delini-Stula, A., and Ortmann, R., Septo-hippocampal system: target for substituted benzamides? Eur. J. Pharmac. *79* (1982) 225–232.

Borowski, E., Ehrl, H., and Starke, K., Relative pre- and postsynaptic potencies of alpha-adrenolytic drugs. Naunyn-Schmiedeberg's Arch. Pharmac., suppl. *293* (1976) R2.

Brown, J., and Handley, S.L., The effects of agents acting at pre- and postsynaptic alpha-adrenoceptors on haloperidol catalepsy. Br. J. Pharmac. *66* (1979) 474P–475P.

Cavero, I., and Roach, A.G., The pharmacology of prazosine, a novel antihypertensive agent. Life Sci. *27* (1980) 1525–1540.

Chapleo, C.B., Doxey, J.C., Myers, P.L., and Roach, A.G., RX781094A, a new potent, selective antagonist of alpha$_2$-adrenoceptors. Br. J. Pharmac. *74* (1981) 842P.

Delini-Stula, A., Baumann, P., and Buech, O., Depression of exploratory activity by clonidine in rats as a model for the detection of relative pre- and postsynaptic central noradrenergic receptor selectivity of alpha-adrenolytic drugs. Naunyn-Schmiedeberg's Arch. Pharmac. *307* (1979) 115–122.

Dettmar, P.W., Lynn, A.G., and Tulloch, I.F., Neuropharmacological evaluation of RX781094A, a new selective alpha$_2$-adrenoceptor antagonist. Br. J. Pharmac. *74* (1981) 843P–844P.

Donaldson, I. McG., Dolphin, A., Jenner, P., Marsden, C.D., and Pycock, C., The roles of noradrenaline and dopamine in contraversive circling behaviour seen after unilateral electrolytic lesions of the locus coeruleus. Eur. J. Pharmac. *39* (1976) 179–191.

Hoffman, B.B., and Lefkowitz, R.J., [^{3}H]-WB4101 – Caution about its role as an alpha-adrenergic subtype selective radioligand. Biochem. Pharmac. *29* (1980) 1537–1541.

Howlett, D.R., Morris, H., and Nahorski, S.R., Anomalous properties of ^{3}H-spiperone binding sites in various areas fo the rat limbic system, Molec. Pharmac. *15* (1979) 506–514.

Leysen, J.E., Gommeren, W., and Laduron, P.M., Spiperone: a ligand of choice for neuroleptic receptors. I. Kinetics and characteristics of in vitro binding. Biochem. Pharmac. *27* (1978) 307–316.

O'Donohue, T.L., Crowley, W.R., and Jacobowitz, D.M., Biochemical mapping of the noradrenergic ventral bundle projection sites: evidence for a noradrenergic-dopaminergic interaction. Brain Res. *172* (1979) 87–100.

Ortmann, R., Bischoff, S., Radeke, E., Buech, O., and Delini-Stula, A., Correlations between different measures of antiserotonergic activity of drugs: study with neuroleptics and 5-HT receptor blockers. Naunyn-Schmiedeberg's Arch. Pharmac. 1982 (in press).

Papeschi, R., and Theiss, P., The effect of yohimbine on the turnover of brain catecholamines and serotonin. Eur. J. Pharmac. *33* (1975) 1–12.

Rochette, L., and Bralet, J., Effect of clonidine on the synthesis of cerebral dopamine. Biochem. Pharmac. *24* (1975) 303–305.

Scatton, B., Zivkovic, B., and Dedek, J., Antidopaminergic properties of yohimbine. J. Pharmac. exp. Ther. *215* (1980) 494–499.

Stroembom, U., Catecholamine receptor agonists. Effects on motor

activity and rate of tyrosine hydroxylation in mouse brain. Naunyn-Schmiedeberg's Arch. Pharmac. *292* (1976) 167-176.
Tanaka, T., Weitzell, R., and Starke, K., High selectivity of rauwolscine for presynaptic alpha-adrenoceptors. Eur. J. Pharmac. *52* (1978) 239-240.
U'Prichard, D.C., and Snyder, S.H., Distinct alpha-noradrenergic receptors differentiated by binding and physiological relationships. Life Sci. *24* (1979) 79-88.
Waldmeier, P.C., Serotoninergic modulation of mesolimbic and frontal cortical dopamine neurons. Experientia *36* (1980) 1092-1094.
Waldmeier, P.C., and Bischoff, S., Interaction of alpha agonists and antagonists with dopaminergic transmission. Experientia *37* (1981) 677.
Waldmeier, P.C., and Maitre, L., On the relevance of preferential increases of mesolimbic vs. striatal dopamine turnover for the prediction of antipsychotic effects of psychotropic drugs. J. Neurochem. *27* (1976) 589-597.
Weitzell, R., Tanaka, T., and Starke, K., Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. Naunyn-Schmiedeberg's Arch. Pharmac. *308* (1979) 127-136.
Westerink, B.H.C., and Korf, J., Regional rat brain levels of 3,4-dihydroxyphenylacetic acid and homovanillic acid: concurrent fluorometric measurement and influence of drugs. Eur. J. Pharmac. *38* (1976) 281-291.

Inactivation of N-assimilating enzymes and proteolytic activities in wheat leaf extracts: Effect of pyridine nucleotides and of adenylates

L. Streit and U. Feller[1]

Pflanzenphysiologisches Institut der Universität Bern, Altenbergrain 21, CH-3013 Bern (Switzerland),
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Summary. Nitrate reductase was protected from inactivation in wheat leaf extracts by NADH, while NADPH was less effective. NAD, NADP or adenylates did not affect nitrate reductase inactivation in vitro. Glutamine synthetase was more stable than nitrate reductase and was protected from inactivation by ATP. ADP, AMP or pyridine nucleotides had no or only a minor effect on the decrease of glutamine synthetase activity in extracts. The caseolytic activity extracted from senescing leaves was slightly decreased by NADH and NADPH but this effect was not sufficient to explain the stabilization of nitrate reductase by NADH. Oxidized pyridine nucleotides and adenylates had no major effect on the caseolytic activity under the conditions used.

Introduction

There is evidence that nitrate reductase (NR) can be inactivated in plant extracts by endogenous peptide hydrolases[2-4]. Phenylmethylsulfonylfluoride (an inhibitor of serine peptide hydrolases) has been found to protect NR from inactivation in extracts from maize roots[5]. Leupeptin (an inhibitor of some endopeptidases) has been noted in barley leaf extracts to prevent the formation of smaller breakdown products from NR[4]. Leupeptin also improves the stability of several enzymes in extracts from castor bean endosperm[6]. Aminopeptidase has been found to be more stable under conditions of low endopeptidase activity in extracts from bean cotyledons collected at various stages of germination[7]. Treatments of the extracts causing lower endopeptidase activities delay the inactivation of aminopeptidase.

For the inactivation of enzymes by proteolysis, both the peptide hydrolase activities present and the susceptibility of the substrate proteins (e.g. enzymes) are important[8]. Substrates, coenzymes and other low molecular weight compounds have been found to change the susceptibility of various enzymes from microorganisms and animals to peptide hydrolases in vitro and in vivo[8]. ATP-stimulated peptide hydrolases have been detected in rat liver[9] and in *E. coli*[10].

Nitrate reductase which is known to be a very unstable enzyme, and glutamine synthetase (GS) are involved in the assimilation of inorganic nitrogen. While the former is needed only for the assimilation of nitrate, GS is also required for the reassimilation of ammonium liberated in the photorespiratory nitrogen cycle[11] or in other metabolic processes (e.g. by phenylalanine ammonia lyase, threonine dehydratase). NR requires for its enzymatic activity reduced pyridine nucleotides and ATP is needed for the GS reaction. In order to investigate the effect of adenylates and of pyridine nucleotides on enzyme inactivation, NR and GS are of interest because of their different cofactor requirements.

The objectives of the present work were to investigate the in vitro stability and inactivation of NR and GS in leaf extracts, and to examine the effects of adenylates and of pyridine nucleotides on the inactivation rates. Since highest endopeptidase activities had been found in wheat leaves late in senescence[12], extracts from senescing flag leaves were used as a source of proteolytic activity.

Materials and methods

Plant materials. Flag leaves of winter wheat (*Triticum aestivum* L., cv. 'Probus') were collected on a field in Zollikofen near Bern at various stages of development during summer 1981. These leaves were transported