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*J Anim Sci* 2001. 79:2202-2209.

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# Complex interaction of ergovaline with 5-HT<sub>2A</sub>, 5-HT<sub>1B/1D</sub>, and alpha<sub>1</sub> receptors in isolated arteries of rat and guinea pig<sup>1</sup>

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**ABSTRACT:** Vascular effects of ergovaline mediated by 5-hydroxytryptamine(HT)<sub>2A</sub>, 5-HT<sub>1B/1D</sub>, and  $\alpha_1$  receptors were studied in isolated arterial preparations of rat and guinea pig. In rat tail artery ergovaline behaved as a potent contractile partial agonist showing an agonist potency (pEC<sub>50</sub>) of  $8.86 \pm 0.03$ , a maximum response (E<sub>max</sub>) of  $59 \pm 2\%$  with respect to 5-HT, and a partial agonist affinity (pK<sub>P</sub>) of  $8.51 \pm 0.06$ . Ergovaline was equipotent with ergotamine (pEC<sub>50</sub>,  $8.69 \pm 0.07$ ; E<sub>max</sub>,  $52 \pm 4\%$ ; pK<sub>P</sub>,  $8.36 \pm 0.11$ ). Contractile responses to ergovaline and ergotamine were surmountably antagonized by the 5-HT<sub>2A</sub> receptor antagonist ketanserin (3 nM). Antagonist affinity (apparent pA<sub>2</sub>) for ketanserin against ergovaline and ergotamine was  $9.19 \pm 0.08$  and  $9.36 \pm 0.17$ , respectively. Ergovaline showed extremely slow on-set and off-set kinetics in rat tail artery. The construction of cumulative concentration-response curves required about 4 h, and the contractile response to ergovaline (30 nM), which completely abolished the subsequent contractile response to 5-HT (10 nM to 1 mM), could not be reversed by wash-out. In guinea pig iliac artery moderately precontracted with

prostaglandin F<sub>2 $\alpha$</sub>  (0.05 to 0.5  $\mu$ M) ergovaline behaved as an agonist (pEC<sub>50</sub>,  $7.71 \pm 0.10$ ) with a potency similar to that of 5-HT (pEC<sub>50</sub>,  $7.60 \pm 0.05$ ). The contractile response to ergovaline was inhibited by the 5-HT<sub>1B/1D</sub> receptor antagonist GR127935 (10 nM). The apparent pA<sub>2</sub> value for GR127935 against ergovaline was  $8.90 \pm 0.12$ . Ergovaline (10 nM) produced no contractile response in guinea pig iliac artery when added before the PGF<sub>2 $\alpha$</sub> -induced precontraction but caused insurmountable blockade of the contractile response to the 5-HT<sub>1B/1D</sub> receptor agonist 5-carboxamidotryptamine (5-CT). The apparent pA<sub>2</sub> value for ergovaline against 5-CT was  $8.56 \pm 0.18$ . In rat thoracic aorta ergovaline (2  $\mu$ M) activated  $\alpha_1$  adrenoceptors only with low efficacy (E<sub>max</sub>,  $12 \pm 3\%$ ) but surmountably antagonized norepinephrine-induced contractions with a pK<sub>P</sub> of  $7.07 \pm 0.12$ . It is concluded that the powerful constrictor effect of ergovaline mediated by activation of vascular 5-HT<sub>2A</sub> and 5-HT<sub>1B/1D</sub> receptors may explain the vascular symptoms of fescue toxicosis observed in livestock grazing tall fescue pastures infected with the endophytic fungus *Neotyphodium coenophialum*.

Key Words: Adrenergic Receptors, Arteries, Ergot Alkaloids, Guinea Pigs, Rats, Serotonin Receptors

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J. Anim. Sci. 2001. 79:2202–2209

## Introduction

There is evidence to suggest that ergopeptide alkaloids are responsible for many health problems of cattle, sheep, and horses feeding on tall fescue (*Festuca arundinacea*) infected with the endophytic fungus *Neotyphodium coenophialum* (Lyons et al., 1986), formerly called *Acremonium coenophialum* (Glenn et al., 1996). Symp-

toms in animals consuming endophyte-infected tall fescue include increased body temperature, loss in average daily weight gain, gangrenous necrosis in extremities ("fescue foot"), low milk production, low blood levels of prolactin, and poor reproductive performance (see Strickland et al., 1993 for review).

Among the ergopeptide alkaloids found in endophyte-infected tall fescue, ergovaline has been reported to be the most abundant, whereas ergotamine, the parent alkaloid of the class to which both compounds belong, is produced only at low levels (Yates et al., 1985). Consequently, ergovaline has been suspected of being a major contributor to the fescue toxicosis syndrome. In contrast to the extensive studies on ergotamine, the pharmacological properties of ergovaline are less well documented (see Oliver, 1997 for review). Ergovaline has been found to be an agonist at cloned rat dopamine D<sub>2</sub> receptors (Larson et al., 1995, 1999) and a partial ago-

<sup>1</sup>The authors wish to thank Dr. W. Schunack for financial support of the study. The generous gifts of drugs by the pharmaceutical companies mentioned in Materials and Methods are gratefully acknowledged.

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Received January 8, 2001.

Accepted April 23, 2001.

nist at 5-HT<sub>2A</sub> receptors in isolated bovine uterine and umbilical arteries (Dyer, 1993).

Evidence has been provided that 5-hydroxytryptamine(HT)<sub>2A</sub> and(or) 5-HT<sub>1B/1D</sub> receptors and  $\alpha_1$ -adrenoceptors mediate contractions in arteries and veins of calves (Dyer, 1993; MacLean et al., 1994), horses (Weller et al., 1994), and sheep (Zhang and Dyer, 1990). Rat assays have previously been established instead of cattle assays to evaluate the toxic components in endophyte-infected tall fescue (Jackson et al., 1989). The aim of the present study was to further characterize the contractile effects of ergovaline in isolated rat and guinea pig arteries that are endowed with 5-HT<sub>2A</sub> receptors, 5-HT<sub>1B/1D</sub> receptors, and  $\alpha_1$ -adrenoceptors.

## Materials and Methods

**Materials.** The following compounds were obtained as gifts: 5-carboxamidotryptamine (**5-CT**; Glaxo Wellcome, Stevenage, U.K.), cimetidine (SmithKline Beecham, Welwyn, U.K.), dinoprost tromethamine (PGF<sub>2 $\alpha$</sub> ; Upjohn, Heppenheim, Germany), GR127935 HCl (2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide; Glaxo Wellcome), ketanserin tartrate (Janssen, Beerse, Belgium), and LY53857 (6-methyl-1-(1-methylethyl)-ergoline-8 $\beta$ -carboxylic acid 2-hydroxyl-1-methylpropyl ester maleate; Eli Lilly and Company, Indianapolis, IN). The following compounds were purchased: carbachol (Sigma, Munich, Germany), cocaine HCl (Merck, Darmstadt, Germany), corticosterone (Sigma), ergotamine tartrate (Research Biochemicals [RBI], Natick, MA), 5-hydroxytryptamine creatinine sulphate (Janssen), idazoxan HCl (RBI), mepyramine hydrogen maleate (Sigma), norepinephrine bitartrate (Merck), prazosin HCl (RBI), (*R,S*)-propranolol (Sigma), and yohimbine HCl (Sigma). Ergovaline tartrate was synthesized in house and its purity was determined by HPLC method published earlier (Shelby et al., 1997). Content of ergovaline in the dry substance was 94.1% and the content of ergovalinine was 0.97%. Ergovaline tartrate was dissolved in ethanol (stock solution 1 mM) and stored at -18°C to minimize hydrolysis and isomerization. Dilutions were freshly prepared in distilled water on the day of the experiment.

**Rat Tail Artery.** Male Wistar rats (280 to 350 g) were killed within an atmosphere of CO<sub>2</sub>. The ventral caudal artery was quickly dissected and cleared of adhering connective tissue. A stainless steel wire (diameter 0.3 mm) was inserted into the artery to rub off the endothelium. Up to 12 cylindrical segments (3 to 4 mm long) were horizontally suspended between two L-shaped stainless steel hooks (diameter 0.15 mm) and mounted in a 20-mL organ bath filled with modified Krebs-Henseleit solution of the following composition (mM): NaCl 118, KCl 4.7, CaCl<sub>2</sub> 2.5, MgSO<sub>4</sub> 1.2, KH<sub>2</sub>PO<sub>4</sub> 1.2, NaHCO<sub>3</sub> 25, and D-glucose 10. The solution was continuously gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub> and warmed to a constant temperature of 37°C (pH 7.4). Preparations

were connected to an isometric force transducer (W. Fleck, Mainz, Germany) attached to a TSE 4711 transducer coupler and a Siemens C 1016 compensograph for the continuous recording of changes in tension. Resting tension was adjusted to 0.5 g at the beginning of each experiment. During an equilibrium period of 120 min, preparations were stimulated once (after 60 min) with 5-HT (1  $\mu$ M). In experiments with ergovaline and ergotamine two cumulative concentration-response curves (**CRC**) were determined on each arterial segment at an interval of 70 min: the first CRC to 5-HT and the second to ergovaline or ergotamine in the absence and presence of the selective 5-HT<sub>2A</sub> receptor antagonist ketanserin (3 nM). Ketanserin was incubated 30 min before the second curve. The shift to the right observed in the presence of ketanserin was calculated considering the shift observed for the respective control preparation in the absence of ketanserin. Because the construction of CRC to ergovaline and ergotamine required about 4 h, two control CRC to 5-HT were additionally determined on the same arterial segment at an interval of 4 h in order to monitor spontaneous changes in agonist sensitivity. In kinetic studies with ergovaline a CRC to 5-HT was determined prior to a bolus application of ergovaline (30 nM). When the contractile response to ergovaline had reached a plateau (usually after 20 min), a second CRC to 5-HT was constructed. After the highest concentration of 5-HT had been administered to the bath fluid, the preparations were washed for 1 h. Prazosin (0.03  $\mu$ M) and cocaine (6  $\mu$ M) were present throughout the experiments to block  $\alpha_1$ -adrenoceptors and neuronal uptake of 5-HT.

**Guinea Pig Iliac Artery.** Guinea pigs of either sex, 300 to 400 g, were killed by cervical dislocation and exsanguination. The abdominal aorta and the right and left common iliac arteries were removed and cleared of adhering connective tissue. Two or three cylindrical segments (1.5 to 2 mm long) from each iliac artery were horizontally suspended between two L-shaped stainless steel hooks (diameter 0.15 mm) and isometrically mounted as described for rat tail artery experiments. The bath fluid (modified Krebs-Henseleit solution [CaCl<sub>2</sub>, 1.25 mM] at 37°C, gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>) contained ketanserin (1  $\mu$ M), prazosin (1  $\mu$ M), idazoxan (1  $\mu$ M), mepyramine (0.3  $\mu$ M), cimetidine (30  $\mu$ M), and cocaine (30  $\mu$ M) to block 5-HT<sub>2A</sub>,  $\alpha_1$ ,  $\alpha_2$ , and histamine H<sub>1</sub>- and H<sub>2</sub>-receptors and neuronal uptake of 5-HT, respectively. The continuous presence of ketanserin (1  $\mu$ M) is of especial importance because the guinea pig iliac artery represents a preparation in which activation of vascular 5-HT<sub>1B/1D</sub> and 5-HT<sub>2A</sub> receptors mediates contraction (Pertz, 1993). The applied resting tension was 0.5 g. During an initial stabilization period of 100 min the bathing medium was changed every 30 min and the tension repeatedly readjusted to 0.5 g. Preparations were then exposed to a near-maximally effective concentration of PGF<sub>2 $\alpha$</sub>  (30  $\mu$ M), which produced a contraction of 1.04  $\pm$  0.05 g ( $n = 27$  vascular segments from six animals). Carbachol (10  $\mu$ M) was

added when the contraction had reached a plateau and preparations were allowed to relax. After washing, re-adjusting of the tension to 0.5 g, and re-equilibration for a further 60 min, the preparations were moderately precontracted with an  $EC_{10}$  to  $EC_{20}$  (0.05 to 0.5  $\mu M$ ) of  $PGF_{2\alpha}$ . The contractile response to ergovaline (1 nM to 1  $\mu M$ ) was studied by constructing a cumulative concentration-response curve in the absence and presence of GR127935 (10 nM). Contractile effects were expressed as a percentage of the  $PGF_{2\alpha}$  (30  $\mu M$ )-induced contraction. GR127935 was incubated for 45 to 60 min. In experiments in which ergovaline was tested as an antagonist of the contractile response to 5-HT, the preparations were precontracted with an  $EC_{10}$  to  $EC_{20}$  of  $PGF_{2\alpha}$  prior to initiating a response to 5-HT (0.3  $\mu M$ ). Two cumulative CRC were determined at an interval of 80 to 90 min on preparations precontracted with an  $EC_{10}$  to  $EC_{20}$  of  $PGF_{2\alpha}$ : the first CRC to 5-HT and the second to 5-HT in the absence and presence of ergovaline (10 nM). Ergovaline was incubated 30 min prior to initiating the precontraction with  $PGF_{2\alpha}$ . In all experiments with antagonists the blocking effects were investigated in arterial segments adjacent to those used as controls.

**Rat Thoracic Aorta.** The thoracic aorta was removed from rats used for studies at 5-HT<sub>2A</sub> receptors in rat tail artery (see above). When cleared of connective tissue the aorta was cut into 6 to 12 segments (3 to 4 mm long). Each segment was rolled with a pair of tweezers to destroy the endothelium. The segments were horizontally suspended between two L-shaped stainless steel holders (diameter 0.3 mm) and mounted isometrically as described for rat tail artery experiments (see above). The bath fluid (modified Krebs-Henseleit solution of the above composition [ $CaCl_2$ , 1.25 mM] at 37°C, gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>) contained yohimbine (0.1  $\mu M$ ), (*R,S*)-propranolol (1  $\mu M$ ), cocaine (10  $\mu M$ ), and corticosterone (30  $\mu M$ ) to block  $\alpha_2$ - and  $\beta$ -adrenoceptors and neuronal and cellular uptake of norepinephrine. The applied resting force was 1 g. During an equilibration period of 3 h the preparations were stimulated three times with norepinephrine (0.3  $\mu M$ ). Two CRC to norepinephrine were determined at an interval of 100 min in the continuous presence of the selective 5-HT<sub>2A</sub> receptor antagonist LY53857 (3  $\mu M$ ), which in contrast to ketanserin has negligible affinity for  $\alpha_1$ -adrenoceptors (Cohen et al., 1983). Ergovaline (2  $\mu M$ ) was incubated 1 h before the second CRC. To establish the validity of this rat model a Schild regression analysis for prazosin (1 to 100 nM) was performed. Schild analysis yielded a straight line with a  $pK_B$  value for prazosin of  $9.65 \pm 0.07$  ( $n = 17$  from four animals). The slope of the Schild plot was  $1.08 \pm 0.08$  (not significantly different from unity). The data are consistent with those estimated by Van der Graaf et al. (1996). In all experiments with antagonists the blocking effects were investigated in arterial segments adjacent to those used as controls.

**Data Presentation and Statistical Evaluation.** Data are presented as means  $\pm$  SEM. Agonist concentration-re-

sponse curves were fitted using the computer program GraphPad Prism 3.0 (GraphPad Software, San Diego, CA). Agonist potencies were expressed as  $pEC_{50}$  values (negative logarithm to base 10 of the molar concentration of the agonist producing 50% of the maximum response). Maximal contractile responses were expressed as  $E_{max}$  values (percentage of the maximal contractile response to a reference compound).

Antagonist affinities of silent antagonists were expressed as either an apparent  $pA_2$  value or a  $pK_B$  value. The apparent  $pA_2$  value was calculated from the following equation:

$$pA_2 = -\log_{10} c(B) + \log_{10} (r - 1)$$

where  $c(B)$  is the molar concentration of antagonist and  $r$  the ratio of agonist  $EC_{50}$  measured in the presence and absence of antagonist (Furchgott, 1972). The  $pK_B$  value was determined according to the method of Arunlakshana and Schild (1959) using antagonist concentrations over 2 log units.

Partial 5-HT<sub>2A</sub> receptor agonist affinities of ergovaline and ergotamine in rat tail artery were estimated according to the method of Kenakin (1993). The equilibrium dissociation constant  $K_P$  for the partial agonist/5-HT<sub>2A</sub> receptor complex was estimated by comparing equiactive molar concentrations of the full agonist  $A$  (5-HT) and the partial agonist  $P$  (ergovaline, ergotamine) in the same arterial segment according to the following equation:

$$c(A) = m \cdot \frac{c(A)}{c(P)} + b$$

with

$$m = \frac{K_P}{\frac{\varepsilon_P}{\varepsilon_A} - 1}$$

where  $c(A)$  is the molar concentration of  $A$ ,  $c(P)$  the molar concentration of  $P$ ,  $m$  the slope, and  $b$  the ordinate intercept of the regression line of  $c(A)$  vs  $c(A)/c(P)$ .  $\varepsilon_A$  and  $\varepsilon_P$  represent the intrinsic efficacies of  $A$  and  $P$ , respectively. If  $\varepsilon_P \ll \varepsilon_A$ ,  $pK_P = -\log_{10} K_P$  can be calculated from this equation:

$$-\log_{10} K_P = \log_{10} m$$

Partial  $\alpha_1$ -adrenoceptor agonist affinity of ergovaline in rat thoracic aorta was estimated according to the method of Marano and Kaumann (1976). The equilibrium dissociation constant  $K_P$  for the partial agonist/ $\alpha_1$ -adrenoceptor complex was estimated by comparing equiactive molar concentrations of the full agonist  $A$  (norepinephrine) in the absence and presence of the partial agonist  $P$  (ergovaline) in the same arterial segment according to the following equation:

$$c(A) = m \cdot c(A)^* + b$$

with

$$m = \frac{1}{1 + \left(1 - \frac{\varepsilon_P}{\varepsilon_A}\right) \cdot \frac{c(P)}{K_P}}$$

where  $c(A)$  is the molar concentration of  $A$  in the absence of  $P$ ,  $c(A)^*$  the molar concentration of  $A$  in the presence of  $P$ ,  $m$  the slope of a weighted regression line of  $c(A)$  vs  $c(A)^*$ , and  $c(P)$  the molar concentration of  $P$ .  $\varepsilon_A$  and  $\varepsilon_P$  represent the intrinsic efficacies of  $A$  and  $P$ , respectively. Weights were calculated according to Marano and Kaumann (1976). If  $\varepsilon_P \ll \varepsilon_A$ ,  $\text{p}K_P = -\log_{10} K_P$  can be calculated from this equation:

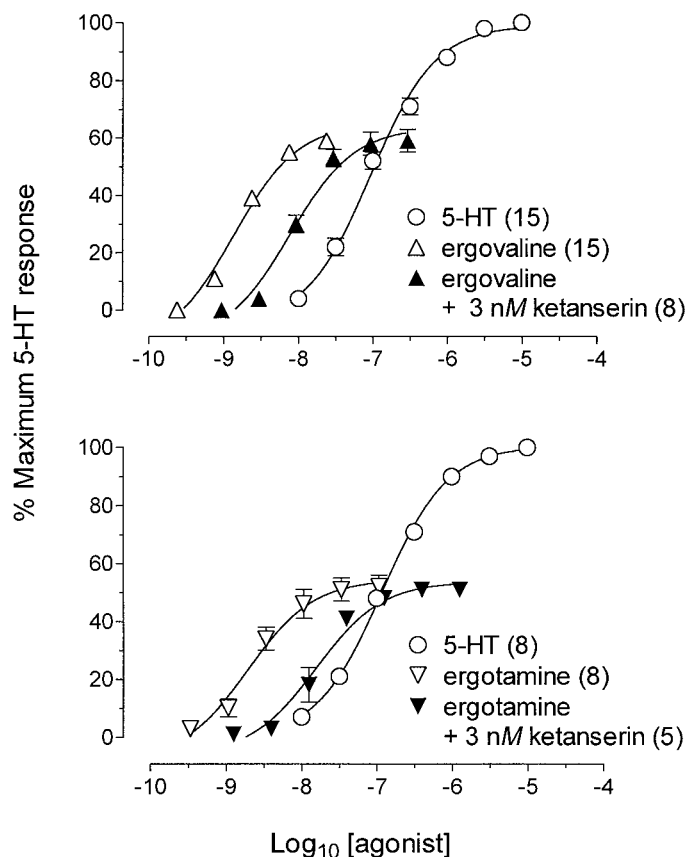
$$\log_{10} \left( \frac{1}{m} - 1 \right) = \log_{10}(P) - \log_{10} K_P$$

Where appropriate, differences between means were determined by Student's  $t$ -test, after checking the homogeneity of the variances;  $P$  values  $< 0.05$  were considered to indicate a significant difference between the responses being compared.

## Results

*Effects of Ergovaline, Ergotamine and 5-HT in Rat Tail Artery.* Ergovaline ( $\text{pEC}_{50} = 8.86 \pm 0.03$ ,  $n = 15$ ), ergotamine ( $\text{pEC}_{50} = 8.69 \pm 0.07$ ,  $n = 8$ ) and 5-HT ( $\text{pEC}_{50} = 7.10 \pm 0.07$ ,  $n = 10$ ) produced concentration-dependent contractile effects in the isolated rat tail artery (Figure 1). Ergovaline and ergotamine were partial agonists relative to 5-HT with  $E_{\text{max}}$  values of  $59 \pm 2$  and  $52 \pm 4\%$  (Figure 1) and partial agonist affinities ( $\text{p}K_P$ ) of  $8.51 \pm 0.06$  and  $8.36 \pm 0.11$ , respectively. The  $\text{pEC}_{50}$  values,  $E_{\text{max}}$  values, and  $\text{p}K_P$  values of ergovaline were not different from those of ergotamine ( $P > 0.05$ ). Construction of CRC to 5-HT required about 15 min, but construction of CRC to ergovaline and ergotamine required about 4 h. Second vs first CRC to 5-HT determined at an interval of 4 h showed a shift to the left of  $0.29 \pm 0.05$  log units and a depression of the maximum response to  $93 \pm 4\%$  ( $n = 10$ ). Thus, ergovaline and ergotamine were approximately 30-fold more potent than 5-HT as contractile agonists as assessed in the rat tail artery bioassay. The 5-HT<sub>2A</sub> receptor antagonist ketanserin (3 nM) produced dextral shifts of the CRC to ergovaline and ergotamine with no depression of the maximum response (Figure 1). The  $\text{p}A_2$  value for ketanserin against ergovaline and ergotamine was  $9.19 \pm 0.08$  ( $n = 8$ ) and  $9.36 \pm 0.17$  ( $n = 5$ ), respectively.

To understand further the kinetics of ergovaline in rat tail artery, we contracted the tissue with 30 nM of the compound for 20 min before initiating a response to 5-HT (10 nM – 1 mM). Ergovaline (30 nM) elicited a contraction of  $72 \pm 4\%$  ( $n = 4$ ) relative to 5-HT and totally abolished the subsequent contractile response to 5-HT (Figure 2). During a following wash-out period



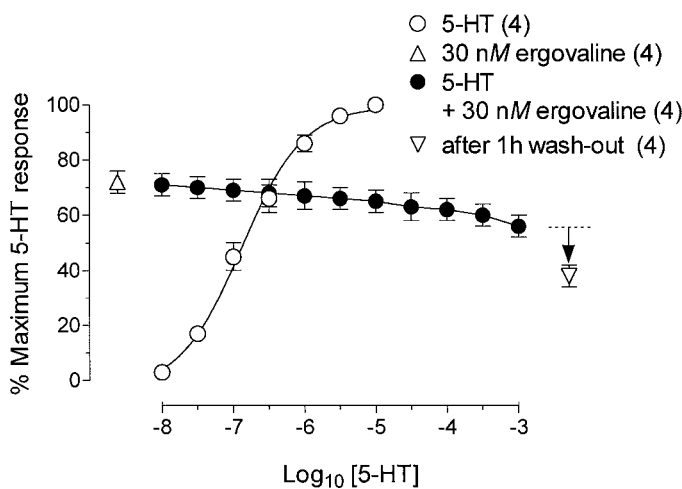
**Figure 1.** Contractile response to 5-HT, ergovaline (upper panel) and ergotamine (lower panel) in rat tail artery. Two successive cumulative concentration-response curves were established at an interval of 70 min: the first curve to 5-HT and the second to ergovaline or ergotamine in the absence and presence of ketanserin (3 nM). Ketanserin was incubated for 30 min. The construction of concentration-response curves to ergovaline and ergotamine required about 4 h. Second 5-HT control curves established 4 h after the first 5-HT control curves showed a shift to the left of  $0.29 \pm 0.05$  log units and a depression of the maximum response to  $93 \pm 4\%$  ( $n = 10$ , not shown). Ordinate: contractions expressed as a percentage of the maximum response to 5-HT observed in the first curve. Abscissa: logarithms to base 10 of molar concentrations of agonists. The data are means  $\pm$  SEM (vertical bars) from four rats. Number of tissues is given in parentheses.

of 60 min the contraction was only slightly diminished and the original baseline was not reached within this time (Figure 2). These observations show that ergovaline dissociates very slowly from the 5-HT<sub>2A</sub> receptor.

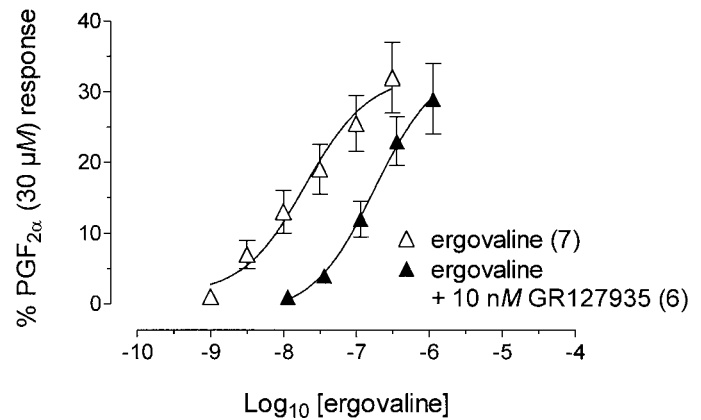
*Effects of Ergovaline in Guinea Pig Iliac Artery.* It has previously been demonstrated that a modest degree of tone in preparations of guinea pig iliac artery induced by a threshold concentration of PGF<sub>2 $\alpha$</sub>  can unmask 5-HT<sub>1B/1D</sub> receptor-mediated contractile responses to 5-HT, 5-carboxamidotryptamine (5-CT) and sumatriptan (Sahin-Erdemli et al., 1991). Ergovaline contracted guinea pig iliac arteries with a  $\text{pEC}_{50}$  of  $7.71 \pm 0.10$  ( $n$

= 7); the  $pEC_{50}$  for 5-HT was  $7.60 \pm 0.05$  ( $n = 11$ ). The maximal agonist effect of ergovaline was  $32 \pm 5\%$  of the contraction induced by  $PGF_{2\alpha}$  ( $30 \mu M$ ) and did not differ ( $P > 0.05$ ) from the maximal agonist effect of 5-HT ( $42 \pm 3\%$ ,  $n = 13$ ). The 5-HT<sub>1B/1D</sub> receptor antagonist GR127935 ( $10 nM$ ) caused a dextral shift of the CRC to ergovaline with no depression of the maximum response (Figure 3). The  $pA_2$  value for GR127935 against ergovaline was  $8.90 \pm 0.12$  ( $n = 6$ ). Based on the observation that agonists can be used as antagonists in guinea pig iliac artery because they do not contract the vessel when added before the  $PGF_{2\alpha}$ -induced precontraction (Schoeffter and Sahin-Erdemli, 1992), the antagonist effect of ergovaline was investigated. Ergovaline ( $10 nM$ ) failed to evoke a contractile response in guinea pig iliac artery when added 30 min prior to a threshold concentration of  $PGF_{2\alpha}$  but caused an insurmountable blockade of the contractile response to the 5-HT<sub>1B/1D</sub> receptor agonist 5-CT (Figure 4). The  $pA_2$  value for ergovaline against 5-CT was  $8.56 \pm 0.18$  ( $n = 5$ ). In contrast to the interaction of ergovaline with 5-HT<sub>2A</sub> receptors in rat tail artery the contractile effects via 5-HT<sub>1B/1D</sub> receptors in guinea pig iliac artery were reversible by wash-out and the original baseline was reached within 5 min.

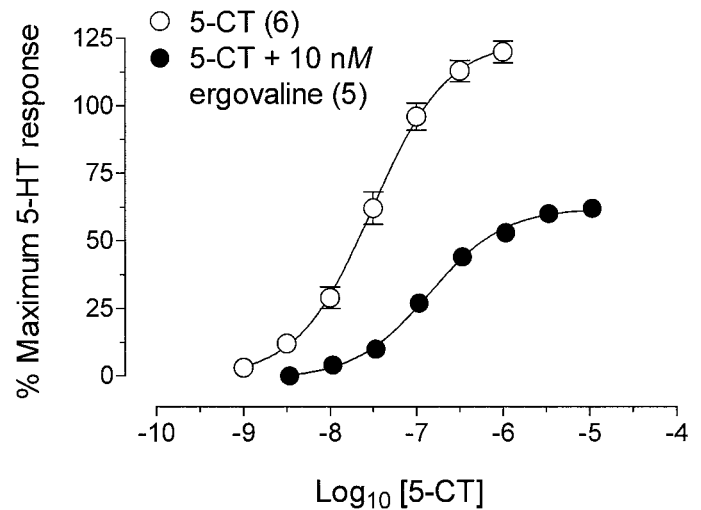
**Effects of Ergovaline in Rat Thoracic Aorta.** Ergovaline ( $2 \mu M$ ) contracted the aorta only with low efficacy ( $E_{max} = 12 \pm 3\%$ ) but surmountably antagonized the contrac-



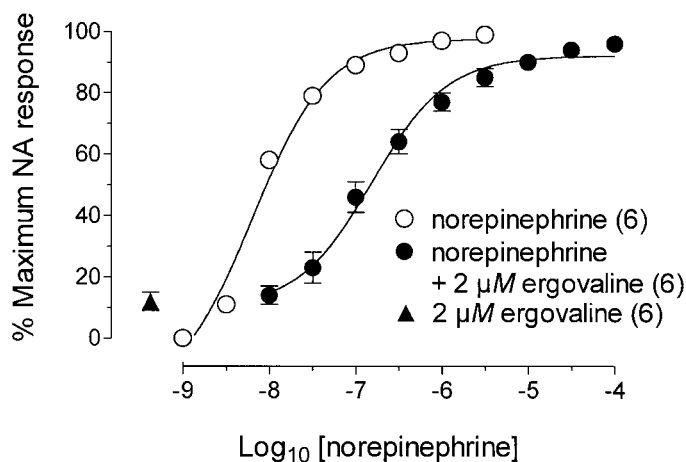
**Figure 2.** Contractile response to 5-HT and to 5-HT 20 min after bolus application of ergovaline in rat tail artery. Two successive cumulative concentration-response curves to 5-HT were established at an interval of 90 min. After the second curve the original baseline was not reached by wash-out within 60 min. Ordinate: contractions expressed as a percentage of the maximum response to 5-HT observed in the first curve. Abscissa: logarithms to base 10 of molar concentrations of 5-HT. Left: stimulant response to ergovaline. Right: contractile effect after 1 h of wash-out. The data are means  $\pm$  SEM (vertical bars) from two rats. Number of tissues is given in parentheses.



**Figure 3.** Contractile response to ergovaline in guinea pig iliac artery moderately precontracted with  $PGF_{2\alpha}$ . Cumulative concentration-response curves were established in the absence and presence of GR127935. GR127935 was incubated for 45 to 60 min. Ordinate: contractions expressed as a percentage of the  $PGF_{2\alpha}$  ( $30 \mu M$ )-induced contraction. Abscissa: logarithms to base 10 of molar concentrations of ergovaline. The data are means  $\pm$  SEM (vertical bars) from three guinea pigs. Number of tissues is given in parentheses.



**Figure 4.** Contractile response to 5-carboxamidotryptamine (5-CT) in guinea pig iliac artery moderately precontracted with  $PGF_{2\alpha}$ . Two successive cumulative concentration-response curves were established at an interval of 80 min: the first curve to 5-HT and the second to 5-CT in the absence and presence of ergovaline. Ergovaline was incubated for 45 to 60 min. Ordinate: contractions expressed as a percentage of the maximum response to 5-HT observed in the first curve. Abscissa: logarithms to base 10 of molar concentrations of 5-CT. The data are means  $\pm$  SEM (vertical bars) from 3 guinea pigs. Number of tissues is given in parentheses.



**Figure 5.** Contractile response to norepinephrine in rat thoracic aorta. Two successive cumulative concentration-response curves to norepinephrine were established at an interval of 100 min. Shown are the second curves in the absence and presence of ergovaline. Ergovaline was incubated for 60 min. Ordinate: contractions expressed as a percentage of the maximum response to norepinephrine observed in the first curve. Abscissa: logarithms to base 10 of molar concentrations of norepinephrine. Left: stimulant response to ergovaline. The data are means  $\pm$  SEM (vertical bars) from three rats. Number of tissues is given in parentheses.

tile response to norepinephrine (Figure 5). The  $pK_P = -\log_{10} K_P$  value was  $7.07 \pm 0.12$  ( $n = 6$ ).

## Discussion

It has been shown in numerous studies that ergot alkaloids possess high affinity for  $\alpha$ -adrenoceptors, dopamine receptors, and nearly all subtypes of 5-HT receptors (see Pertz and Eich, 1999 for review). The clinically effective ergopeptide alkaloid ergotamine activates 5-HT<sub>2A</sub> receptors (Kalkman and Schneider, 1996) and may act on 5-HT<sub>1B/1D</sub> receptors and  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors as an agonist and (or) antagonist depending on the vascular bed (Müller-Schweinitzer, 1983, 1992; Silberstein, 1997 for review).

The experiments of the present study in rat tail artery confirm and extend previously published findings in bovine uterine and umbilical arteries that 5-HT<sub>2A</sub> receptors mediate ergovaline-induced contractions of blood vessels (Dyer, 1993). Agonist potency and maximal contractile response of ergovaline in rat tail artery were in the same range as determined in bovine uterine and umbilical arteries (Dyer, 1993). Ergovaline was as effective as ergotamine as a partial agonist in rat tail artery. Partial agonist affinities ( $pK_P$  values) of ergovaline and ergotamine were in reasonable agreement with their agonist potencies ( $pEC_{50}$  values). The  $pA_2$  value for the potent 5-HT<sub>2A</sub> receptor-selective antagonist ketanserin against ergovaline (9.2) and ergotamine (9.4)

was close to a  $pA_2$  value of 9.5 for ketanserin against 5-HT (Pertz et al., 1999). The similarity of blocking potency of ketanserin against ergovaline, ergotamine, and 5-HT is consistent with the interaction of the three drugs with the same receptor class (5-HT<sub>2A</sub>) in this tissue. It should be mentioned that in the study of Dyer (1993) the blocking potency of ketanserin against ergovaline could not be quantified because the contractile response to only a single concentration of ergovaline (10 nM) was inhibited by ketanserin (30 to 100 nM).

Ergovaline- and ergotamine-mediated contractions in rat tail artery were slow in onset and the construction of cumulative CRC required about 4 h. The observation is consistent with previously reported findings in bovine uterine and umbilical arteries, in which low concentrations of ergovaline required a minimum of 120 min to reach the maximum response (Dyer, 1993). The ability of the partial agonist ergovaline (30 nM) to completely abolish the contractile response to 5-HT (Figure 2) illustrates that the ergopeptide derivative can bind to the 5-HT<sub>2A</sub> receptor in an irreversible manner. Dissociation from the receptor is so slow that neither 5-HT administration nor subsequent wash-out are capable of reversing the occupation of the receptor by ergovaline. Long-lasting biological effects are well-known from ergotamine (Silberstein, 1997), and metergoline has been shown to act as a slow on-set and off-set 5-HT<sub>2A</sub> receptor antagonist of 5-HT-induced vasoconstrictor responses in the isolated, perfused rat kidney (Bond et al., 1989). It should be emphasized that the kinetic properties of ergovaline are a crucial point in the medical treatment of "fescue foot" in animals consuming endophyte-infected tall fescue. The decreased peripheral blood flow induced by the powerful constrictor effects of ergovaline via activation of vascular 5-HT<sub>2A</sub> receptors can only be prevented by prior administration of a 5-HT<sub>2A</sub> receptor antagonist such as ketanserin (see Figure 1) but cannot readily be reversed after signs of "fescue foot" have appeared. For the sake of completeness it should be mentioned that the quick wash-out of ergovaline observed in guinea pig iliac artery needs to be interpreted with caution because not only ergovaline is removed by wash-out, but also PGF<sub>2 $\alpha$</sub> , which is required for agonists (e.g., ergovaline) to induce contraction. It is difficult to determine whether ergovaline has still been bound to 5-HT<sub>1B/1D</sub> receptors of guinea pig iliac artery.

Ergovaline did not only contract rat tail arteries via activation of vascular 5-HT<sub>2A</sub> receptors, but also elicited contraction of guinea pig iliac arteries via activation of vascular 5-HT<sub>1B/1D</sub> receptors. Ergovaline ( $pEC_{50} = 7.7$ ) was equipotent with 5-HT ( $pEC_{50} = 7.6$ ) in this tissue. The  $pA_2$  value for GR127935 against ergovaline (8.9) was identical to the affinity value  $pK_i$  reported for this antagonist at cloned guinea pig 5-HT<sub>1D</sub> receptors (Wurch et al., 1997). The experiments with ergovaline as an antagonist of the contractile response to the potent 5-HT<sub>1B/1D</sub> receptor agonist 5-CT were conducted to provide further evidence that the ergopeptide derivative interacts with this subtype of 5-HT receptors. Inter-

estingly, some ergolines, such as metergoline, methysergide, and ergotamine, have been alternatively described as antagonists or (partial) agonists in various 5-HT<sub>1B/1D</sub> receptor models (Müller-Schweinitzer, 1983; Schoeffter and Sahin-Erdemli, 1992). The experiments of the present study show that ergovaline may act as an agonist and/or antagonist at 5-HT<sub>1B/1D</sub> receptors depending on the conditions and models used (Hoyer and Boddeke, 1993). It should be noted that the affinity of ergovaline ( $pA_2$  8.6) was somewhat higher than its agonist potency ( $pEC_{50}$  7.7). The observation indicates that  $pEC_{50}$ , in contrast to  $pA_2$  or  $pK_i$ , values do not necessarily reflect affinity values (Kenakin, 1988).

Dyer (1993) reported that the nonselective  $\alpha_1/\alpha_2$ -adrenoceptor antagonist phentolamine and the selective  $\alpha_1$ -adrenoceptor antagonist prazosin failed to block ergovaline-induced contractions in bovine uterine artery. The observation indicated that  $\alpha$ -adrenoceptors are not involved in the vasoconstrictor response to ergovaline. At  $\alpha_1$ -adrenoceptors of rat thoracic aorta ergovaline proved to be a low-efficacy partial agonist. As expected for a classic partial agonist, ergovaline caused surmountable antagonism of the contractile response to norepinephrine (Figure 5). The affinity value  $pK_P$  of ergovaline at  $\alpha_1$ -adrenoceptors (7.1) was lower than the affinity value  $pK_P$  at 5-HT<sub>2A</sub> receptors (8.5) and the affinity value  $pA_2$  at 5-HT<sub>1B/1D</sub> receptors (8.6). In addition, the maximal agonist effects of ergovaline were substantially higher at 5-HT<sub>1B/1D</sub> and 5-HT<sub>2A</sub> receptors compared to those at  $\alpha_1$ -adrenoceptors. The present study provides further evidence of serotonergic mechanisms involved in the vascular symptoms associated with fescue toxicosis.

Based on available evidence, activation of 5-HT<sub>2A</sub> receptors by ergovaline may also be responsible for the increased body temperature observed in animals consuming endophyte-infected tall fescue. Hyperthermic responses to 5-HT receptor agonists are mediated by 5-HT<sub>2A</sub> or 5-HT<sub>2C</sub> receptors (Gudelsky et al., 1987). Unfortunately, there are no data available for the interaction of ergovaline with 5-HT<sub>2C</sub> receptors. Because 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptors possess a high degree of pharmacological similarity (Baxter et al., 1995) and ergolines generally are drugs of low selectivity (see Pertz and Eich, 1999 for review), ergovaline may also be a potent 5-HT<sub>2C</sub> receptor agonist. We found comparably high agonist activities for ergovaline and ergotamine at 5-HT<sub>2A</sub> receptors (Figure 1), and for ergotamine potent 5-HT<sub>2C</sub> receptor agonism has previously been demonstrated (Brown et al., 1991). Alternatively, an indirect mechanism may underlie the hyperthermic response to ergovaline due to the powerful vasoconstrictor effects of the ergopeptide derivative via activation of vascular 5-HT<sub>2A</sub> and 5-HT<sub>1B/1D</sub> receptors. The reduction in blood flow to peripheral tissues may have the consequence that animals are less efficient in cooling themselves (Cross, 1997). Due to its 5-HT<sub>2A</sub> receptor agonist activity ergovaline may also be responsible for the reduced appetite of animals feeding on endophyte-in-

fectured tall fescue, because peripheral 5-HT<sub>2A</sub> receptor agonists have been shown to suppress food intake (Sugimoto et al., 1996). A further symptom in fescue toxicosis is a decrease in milk production, which is most likely explained by decreased serum prolactin (Strickland et al., 1993). The low blood levels of prolactin observed in animals consuming endophyte-infected tall fescue have been attributed to ergovaline due to its dopamine D<sub>2</sub> receptor agonist activity (Larson et al., 1995). However, it has recently been reported that 5-HT<sub>2A</sub> receptors are involved in the regulation of prolactin release (Albinsson et al., 1994). Further experiments are required to clarify the question of whether the partial agonist ergovaline can induce a decrease in serum prolactin due to its 5-HT<sub>2A</sub> receptor blocking properties. Recent data favor this hypothesis; ergovaline showed low affinity for cloned human dopamine D<sub>2</sub> ( $K_i = 250 \pm 40$  nM) and D<sub>3</sub> receptors ( $K_i = 540 \pm 60$  nM), in contrast to ergotamine (D<sub>2</sub>:  $K_i = 1.6 \pm 0.6$  nM; D<sub>3</sub>:  $K_i = 7.5 \pm 2$  nM) (P. Sokoloff, personal communication).

## Implications

Ergovaline exerts a deleterious effect in the vasculature mainly due to its powerful constrictor response via activation of vascular 5-HT<sub>2A</sub> and 5-HT<sub>1B/1D</sub> receptors. Dependent on the vascular bed,  $\alpha_1$ -adrenoceptors may also be involved. The slow on-set and off-set kinetics of ergovaline at vascular 5-HT<sub>2A</sub> receptors complicates the development of treatment protocols to reduce or eliminate fescue toxicosis in livestock. The prophylactic administration of a mixed antagonist for both 5-HT<sub>2A</sub> and 5-HT<sub>1B/1D</sub> receptors might be effective.

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