Mediation of the hypotensive action of systemic clonidine in the rat by α$_2$-adrenoceptors

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1 During the past few years it has been shown that the sympatholytic effect resulting from localized microinjection of clonidine and other imidazolines into the rostral ventrolateral medulla (RVL) results from activation of 'imidazoline' receptors (I$_1$ receptors) rather than from an α$_2$-adrenoceptor-mediated effect.

2 The relative contributions of these two receptor systems to the hypotensive action of systemically administered clonidine have not been studied. Clonidine has afffinity for both I$_1$ and α$_2$-adrenoceptors; guanabenz represents a useful pharmacological tool, since it activates only the α$_2$-adrenoceptor.

3 Antagonists acting at both I$_1$ and α$_2$-adrenoceptors (idazoxan) and at only α$_2$-adrenoceptors (SK&F 86466; 6-chloro-3-methyl-2,3,4,5-tetrahydro-3-benzazepine) are available. Idazoxan (1 mg kg^{-1}, i.v.) and SK&F 86466 (3 mg kg^{-1}, i.v.) produced an equivalent degree of blockade of the pressor response to guanabenz or clonidine in the pithed rat, a response mediated by the α$_2$-adrenoceptor.

4 Guanabenz (30 µg kg^{-1}, i.v.) and clonidine (10 µg kg^{-1}, i.v.) lowered blood pressure in the chloralose-anaesthetized spontaneously hypertensive rat by 48 ± 4.6 mmHg and 44 ± 5.4 mmHg, respectively; this response, for either agonist, was blocked by both idazoxan and SK&F 86466.

5 These data show that the hypotensive effect of intravenously administered clonidine results from activation of central α$_2$-adrenoceptors, with no significant contribution from an I$_1$-mediated effect. Thus clonidine can lower blood pressure by different receptor mechanisms, dependent on the route of administration.

Keywords: α$_2$-Adrenoceptors; imidazoline receptors; idazoxan; guanabenz; SK&F 86466; centrally acting antihypertensives; rostral ventrolateral medulla

Introduction

It is now recognized that there are specific 'receptors' which recognize compounds containing imidazole, imidazoline and guanidinum moities, although the endogenous ligand(s) for these receptors has not yet been identified (Ernsberger et al., 1987; Parini et al., 1989; Lehmann et al., 1989; Bricca et al., 1989a; Michel et al., 1989; Wikberg & Uhlen, 1990). Most of the characterization of these receptors has been performed via radioligand binding assays, and two major subtypes of imidazoline receptor have been identified, and designated I$_1$ and I$_2$ (Michel & Ernsberger, 1992). The I$_1$ receptor is preferentially labelled by [^{3}H]-clonidine or [^{3}H]-para-aminoclonidine, and the I$_2$ by [^{3}H]-idazoxan. In both man and experimental animals, a high density of I$_1$ receptors is found in a specific area of the brainstem, the rostral ventrolateral medullar (RVL; also referred to as the nucleus reticularis lateralis (NRL)) (Ernsberger et al., 1987; Bricca et al., 1989b), and, in the rat, the hypotensive and bradycardiac action of clonidine and other imidazolines, administered via localized microinjection to this region, has been shown to result from activation of I$_1$ receptors (Ernsberger et al., 1990; 1992; Gomez et al., 1991). Thus, the hypotensive action of clonidine could be antagonized by local injection of idazoxan, an antagonist having affinity for both I$_1$ receptors and α$_2$-adrenoceptors, but not by SK&F 86466 (6-chloro-3-methyl-2,3,4,5-tetrahydro-3-benzazepine) which blocks only the α$_2$-adrenoceptor. Guanabenz, an agonist at α$_2$-adrenoceptors but not at I$_1$ receptors, had little effect on blood pressure and heart rate when administered to the RVL (Ernsberger et al., 1990).

It has long been assumed that the ability of clonidine and related compounds to lower blood pressure results from stimulation of central α$_2$-adrenoceptors. The selective α$_2$-adrenoceptor antagonists, yohimbine and rauwolscine, will produce dose-related blockade of the hypotensive effect of clonidine, administered via the vertebral artery to the anaesthetized cat (Timmermans et al., 1981). Similar results are observed in the dog (Schmitt et al., 1973). Intravenous yohimbine has been shown to block the hypotensive action of intravenous clonidine in the anaesthetized normotensive rat (Gutkind et al., 1986). In the anaesthetized cat (Hamilton et al., 1980) or rat (Borkowski & Finch, 1979), centrally administered yohimbine can block the hypotensive response to intracerebroventricular clonidine. Since yohimbine has essentially no affinity for the I$_1$ receptor (Ernsberger et al., 1987; Bricca et al., 1989a), it is likely that clonidine is acting via α$_2$-adrenoceptors in the above models. However, a recent study (Tibirica et al., 1991a) found a low dose of intracisternal yohimbine to be ineffective against the hypotensive effect of intravenous clonidine, although the clonidine-induced metabolic effects in the locus coeruleus were attenuated.

In this report, we examine the relative role of α$_2$-adrenoceptors and I$_1$-receptors in the blood pressure lowering effect of intravenous clonidine in the anaesthetized spontaneously hypertensive rat.

Methods

Measurement of α$_2$-adrenoceptor-mediated pressor activity in the pithed rat

Male Sprague-Dawley rats (Charles River Labs, Wilmington, Mass., U.S.A.) weighing 250–350 g were anaesthetized with methohexitone (10 mg kg^{-1}, i.v.). The trachea was cannulated and polyethylene cannulae containing heparinized (20 units ml^{-1}) saline were introduced into the femoral artery and vein for monitoring blood pressure and intravenous drug administration, respectively. One orbit was perforated and the spinal cord destroyed by pushing a stainless steel rod (2 mm dia-

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meter) forward through the spinal canal. Immediately after pithing, the rats were connectd to a rodent respirator (Harvard Model No. 683) via the tracheal cannula, and artificially respired at 60 strokes min^{-1}, with a tidal volume of 1 ml 100 g^{-1} of body weight. Animals were gently secured to a water-circulating heating pad maintained at 37°C.

Clonidine (0.1–100 µg kg^{-1}) or guanabenz (1.0–300 µg kg^{-1}) were administered serially via i.v. bolus. Changes in blood pressure were monitored via the arterial cannula with a pressure transducer and physiological recorder. Doses were administered when the blood pressure response to the previous dose had stabilized. Only one dose-response curve was determined in each animal. Test antagonists were administered via slow i.v. bolus (1–2 min) 5 min prior to determination of the agonist dose-response curve.

Measurement of blood pressure effects in anaesthetized rats

Male spontaneously hypertensive rats (Taconic Farms, Germantown, NY, U.S.A.), age 16–20 weeks, were anaesthetized with α-chloralose (80 mg kg^{-1}, i.v.) and placed on a water-circulating heating pad maintained at 37°C. Polyethylene cannulae containing heparinized (20 units ml^{-1}) saline were inserted into the femoral artery and vein.

Animals were given a single i.v. bolus dose of either clonidine (10 µg kg^{-1}) or guanabenz (30 µg kg^{-1}) and blood pressure was monitored for 70 min. Test antagonists (SK&F 86466 or idazoxan) were administered via slow (1–2 min) i.v. bolus injetion 5 min before agonist challenge.

Drugs used

Clonidine, guanabenz and idazoxan were obtained from Research Biochemicals Inc (Natick, Mass, U.S.A.). α-Chloralose was obtained from Fisher Scientific Company. SK&F 86466   (6-chloro-3-methyl-2,3,4,5-tetrahydro-3-benzazepine) was synthesized at SmithKline Beecham Pharmaceuticals.

Results

Both clonidine and guanabenz produced dose-related increases in blood pressure in the pithed normotensive rat (Figures 1 and 2). Clonidine was somewhat more potent ($ED_{50} = 3$ µg kg^{-1}) and produced a slightly greater maximum increase in diastolic blood pressure (108 mmHg) than guanazbenz ($ED_{50} = 20$ µg kg^{-1}; maximum response = 95 mmHg). SK&F 86466, at a dose of 3 mg kg^{-1}, i.v., produced a 5.2
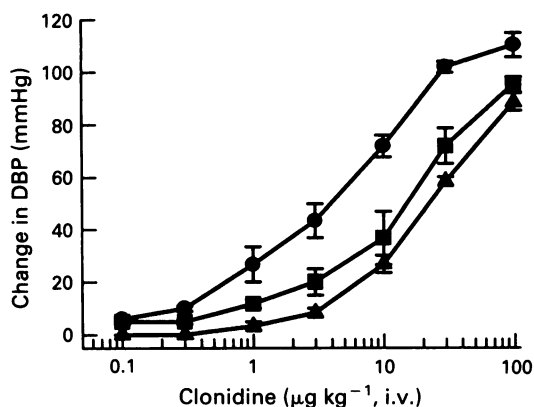
fold shift to the right in the clonidine dose-response curve, idazoxan, at a dose of 1 mg kg^{-1}, i.v., produced a 3.6 fold shift (Figure 1). These antagonists produced a slightly greater shift in the dose-response curve for guanabenz (8.7 and 10 fold shifts for idazoxan and SK&F 86466, respectively at the same doses tested against clonidine) (Figure 2).

In chloralose-anaesthetized spontaneously hypertensive rats, clonidine (10 µg kg^{-1}, i.v.) produced its well known biphasic effect on blood pressure, with an initial increase, followed by a long lasting fall in pressure. The hypotensive phase was attenuated by SK&F 86466 at 1 mg kg^{-1}, i.v., administered 5 min prior to clonidine. Increasing the SK&F 86466 dose to 3 mg kg^{-1} resulted in a nearly complete abolition of the hypotensive phase, and a substantial attenuation of the initial pressor response (Figure 3). Similar results were obtained with guanabenz (30 µg kg^{-1}; Figure 4). Figure 5 compares the ability of idazoxan and SK&F 86466 to block the antihypertensive action of clonidine and guanabenz. It is clear that the two agonists are equally sensitive to both idazoxan and SK&F 86466. Furthermore treatment with idazoxan (1 mg kg^{-1}) or SK&F 86466 (3 mg kg^{-1}) produces an equivalent degree of blockade, as measured by attenuation of the peak antihypertensive effect.
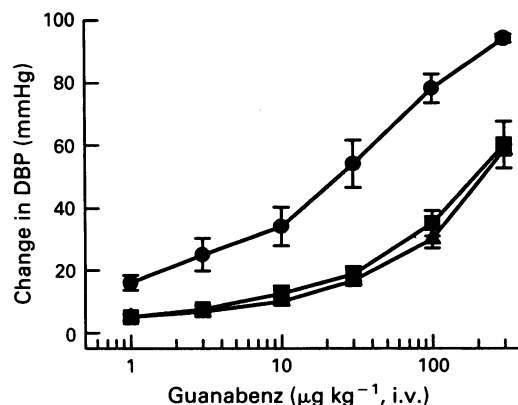
**Figure 2** Effect of idazoxan (1 mg kg^{-1}, i.v., ■) and SK&F 86466 (3 mg kg^{-1}, i.v., ▲) on the pressor response to guanabenz in the pithed rat; control (●). Response to guanabenz expressed as increase in diastolic blood pressure (DBP). Basal DBP (mean of treatment groups) = 41 ± 2 mmHg. Each curve represents the mean ± s.e.mean of six animals.

Figure 1 Effect of idazoxan (1 mg kg^{-1}, i.v., ■) and SK&F 86466 (3 mg kg^{-1}, i.v., ▲) on the pressor response to clonidine in the pithed rat; control (●). Response to clonidine expressed as increase in diastolic blood pressure (DBP). Basal DBP (mean of treatment groups) = 42 ± 3 mmHg. Each curve represents the mean ± s.e.mean of six animals.
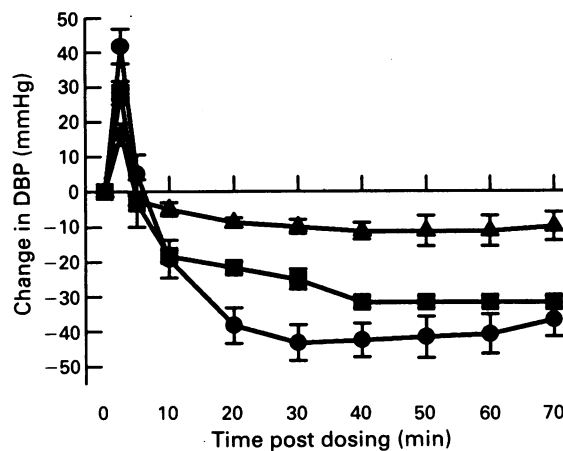
Figure 3 Blockade by SK&F 86466 of the antihypertensive action of clonidine (10 µg kg^{-1}) in the chloralose-anaesthetized spontaneously hypertensive rat. Clonidine administered in time = 0, in SK&F 86466-treated animals, the antagonist was administered 5 min prior to clonidine challenge. All drugs were given via i.v. bolus administration: (●) control; (■) 1 mg kg^{-1} SK&F 86466; (▲) 3 mg kg^{-1} SK&F 86466. Each curve represents the mean ± s.e.mean of six animals.
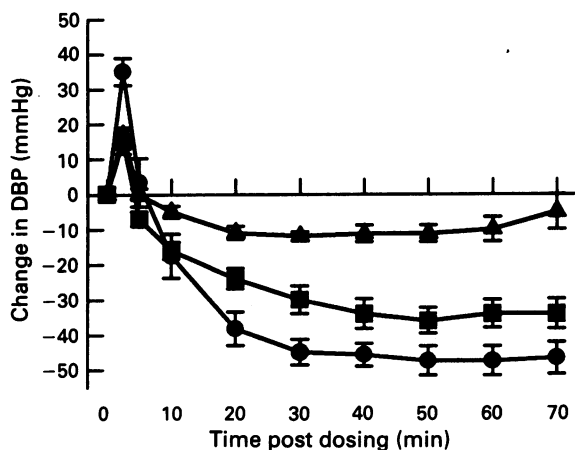
Figure 4 Blockade by SK&F 86466 of the antihypertensive action of guanabenz ($30\,\mu g\,kg^{-1}$) in the chloralose-anaesthetized spontaneously hypertensive rat. Guanabenz administered at time = 0; in SK&F 86466-treated animals, the antagonist was administered 5 min prior to clonidine challenge. All drugs were given via i.v. bolus administration. Symbols as in Figure 3. Each curve represents the mean ± s.e.mean of six animals.
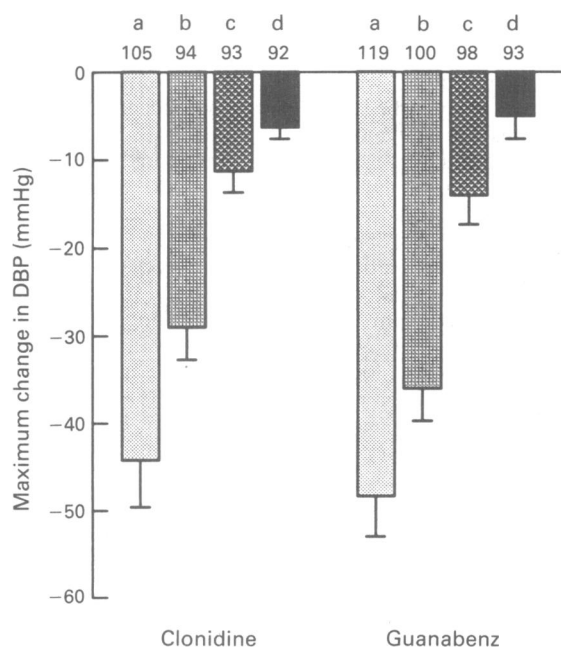
Figure 5 Effect of SK&F 86466 and idazoxan on the antihypertensive action of clonidine ($10\,\mu g\,kg^{-1}$) and guanabenz ($30\,\mu g\,kg^{-1}$) in the chloralose-anaesthetized rat. All drugs were administered via i.v. bolus. Each column represents the maximum decrease in diastolic blood pressure (DBP) observed during the 70 min interval following agonist administration (see Figures 3 and 4 for the time course of the response to clonidine and guanabenz, respectively). Basal DBP prior to antagonist administration was 108.5 ± 2.5 mmHg (mean of all groups). DBP subsequent to antagonist dosing, but prior to agonist challenge is shown above each column. (a) Control; (b) SK&F 86466 $1\,mg\,kg^{-1}$; (c) SK&F 86466 $3\,mg\,kg^{-1}$; (d) idazoxan $1\,mg\,kg^{-1}$. Each column represents the mean with s.e.mean of six animals.

Discussion

The evidence for involvement of I_1 receptors in the sympatholytic action of clonidine, administered by localized injection to the RVL region of the rat, is quite convincing. Indeed, since the hypotensive effect of clonidine is insensitive

to SK&F 86466, and the ability of a structurally diverse series of compounds to lower blood pressure and heart rate correlates well with I_1 receptor, but not with α_2-adrenoceptor affinity (Ernsberger et al., 1990) it appears that the α_2-adrenoceptor may not be involved to any significant degree when agonists are administered via this route.

On the other hand, the early studies showing blockade by yohimbine or rauwolscine of the effects of clonidine, administered via the intravertebral or intra-cerebroventricular route, support an α_2-adrenoceptor-mediated effect. At least in some cases (Borkowski & Finch, 1979) the yohimbine doses are high, and it is known that high doses of yohimbine can block α_1-adrenoceptors as well as other neurotransmitter receptors (e.g. 5-hydroxytryptamine (5-HT) and dopamine). It is unlikely that clonidine would lower blood pressure via activation of 5-HT or dopamine receptors. Although a hypotensive action mediated via central α_1-adrenoceptors has been suggested (Gutkind et al., 1986), the studies of Timmermans et al. (1981) using yohimbine, rauwolscine and corynanthine suggest that clonidine does not act via this mechanism, at least in the cat.

Clonidine, which crosses the blood brain barrier readily, will have access to many sites within the central nervous system upon intravenous or intra-vertebral arterial administration. It is known that blood pressure can be reduced by α-adrenoceptor stimulation in locations other than the RVL, including the nucleus tractus solitarius (NTS) (Kubo & Misu, 1981; Brody et al., 1984). It also appears that different agonists, each presumed to be acting at α_2-adrenoceptors, can lower blood pressure by acting at different sites within the central nervous system (Scholtysik et al., 1975; Gutkind et al., 1986; van den Buuse et al., 1993). Gutkind et al. (1986) found the hypotensive action of clonidine, but not guanabenz to be sensitive to yohimbine blockade. These results differ from our data, obtained with different antagonists, which suggest that these agonists act via a similar mechanism.

Considering the multiple pathways controlling sympathetic outflow, it is likely that different receptor mechanisms could be involved in the generalized central application of clonidine from when the drug is locally applied to a specific nucleus. Intracerebroventricular administration may represent an intermediate situation, since access may depend on the distance of individual nuclei from the ventricular surface. It is even possible that different receptor mechanisms are involved in conscious and anaesthetized animals, since intracerebroventricular yohimbine failed to block the hypotensive effect of intracerebroventricular clonidine in conscious normotensive or hypertensive rats (Kawasaki et al., 1992), contrasting to similar experiments in anaesthetized animals where the same intracerebroventricular dose of yohimbine was effective (Borkowski & Finch, 1979).

Functional and radioligand binding studies suggest that the agonists and antagonists used in this study should be suitable tools for evaluating the relative contributions of α_2-adrenoceptors and I_1 receptors. Table 1 shows representative values showing clonidine and idazoxan to have affinity for both receptors, and guanabenz and SK&F 86466 to be highly selective for the α_2-adrenoceptor. Guanabenz was found to be slightly more potent than clonidine at α_2-adrenoceptors in the RVL. Radioligand binding studies at this receptor in rat, canine and bovine cortex have shown either agent to be more potent, although the potency ratio is generally two or less (Table 1). Consistent with our data (Figures 1 and 2), guanabenz has been reported to be slightly less potent than clonidine as a pressor agent in the pithed rat, with a lower maximum response (Gutkind et al., 1986). Guanabenz is also less potent than clonidine in producing hypotension and bradycardia in the anaesthetized rat (Gutkind et al., 1986) and as an antihypertensive drug in man (Walker et al., 1982). SK&F 86466 has about ten fold lower α_2-adrenoceptor affinity than idazoxan in the bovine RVL (Ernsberger et al., 1990); however, in other α_2-adrenoceptor binding assays, the two compounds have essentially equal

Table 1 Receptor affinities of pharmacological tools for differentiation of α_2-adrenoceptors and I_1 receptors

	Affinity (nM)[1]				
Tissue	*Clon*	*GBZ*	*SKF*	*IDZ*	*Reference*
α_2-*Adrenoceptors*					
Human adipocyte	32	39	26	19	Langin *et al.*, 1990
Bovine RVL[2]	28	7.2	35	3.6	Ernsberger *et al.*, 1990
Bovine cortex	5.3	11	84	61	Ernsberger *et al.*, 1990
Guinea-pig ileum[3]	8.5	14	300	31	Hieble *et al.*, 1990
Guinea-pig atrium[4]	15	ND	17	13	Hieble *et al.*, 1986a,b
I_1-*receptors*					
Bovine RVL[5]	1	$>10^6$	93,000	186	Ernsberger *et al.*, 1990

[1]Affinity of clonidine (Clon), guanabenz (GBZ), SK&F 86466 (SKF) and idazoxan (IDZ) expressed as K_i for inhibition of radioligand binding, K_B (functional potency of antagonists) or IC_{50} (functional potency of agonists). Unless otherwise noted, affinity determined by radioligand binding assay.
[2]α_2-Adrenoceptor component of [^{3}H]-p-amino clonidine binding.
[3]Functional potency for inhibition of short circuit current, or blockade of the inhibitory effect of clonidine.
[4]Functional potency for inhibition of the inotropic response to field stimulation, or blockade of the inhibitory effect of B-HT 920.
[5]I_1 receptor component of [^{3}H]-p-amino clonidine binding.

affinity (Ernsberger *et al.*, 1990; Blaxall *et al.*, 1991), and functional *in vitro* assays show the two antagonists to be essentially equipotent (Hieble *et al.*, 1986a,b).

Our *in vivo* assay in the pithed rat shows idazoxan to be about three fold more potent as an α_2-adrenoceptor antagonist, as reflected by blockade of guanabenz-induced increases in blood pressure (Figure 2). The lower absolute potency of both SK&F 86466 and idazoxan against the response to clonidine in this model may reflect the greater contribution of the α_1-adrenoceptor to the clonidine-induced pressor response, consistent with previous data in the pithed rat (Timmermans & Van Zweiten, 1980; Gutkind *et al.*, 1986). Clonidine is less selective than guanabenz as an α_2-adrenoceptor agonist, based on ability to inhibit binding of antagonist ligands to central α_1- and α_2-adrenoceptors (Megens *et al.*, 1986).

In contrast to α-adrenoceptor-mediated pressor responses in the pithed rat, the hypotensive response to clonidine could be mediated either by α_2-adrenoceptors, I_1-receptors, or by a combination of the two mechanisms. Since SK&F 86466 was essentially equipotent against clonidine and guanabenz, it is unlikely that an I_1 receptor-mediated effect makes a significant contribution to the hypotensive response to intravenous clonidine.

It has been suggested that clonidine and guanabenz may lower blood pressure in the rat by an action at different centres within the central nervous system (Gutkind *et al.*, 1986). Based on the different *in vitro* affinities of these two

agonists for I_1 receptors, this certainly remains a possibility. Studies with local administration of a variety of centrally acting sympatholytic agents supports differences in their mechanism of action. Nevertheless the current results would suggest that both clonidine and guanabenz act by stimulation of α_2-adrenoceptors when administered intravenously, although it cannot be established that identical neuronal populations are activated.

Several agents, e.g. rilmenidine and moxonidine, have been postulated to have greater selectivity than clonidine for I_1 receptors versus α_2-adrenoceptors (Bricca *et al.*, 1989b; Gomez *et al.*, 1992; Ernsberger *et al.*, 1992; 1993). It has been proposed that the relative lack of side effects of these drugs, *vis-à-vis* clonidine and other clinically used α_2-adrenoceptor agonists, results from this selectivity (King *et al.*, 1992; Tibirica *et al.*, 1991b). Alternatively, some other difference in the overall pharmacological profile could contribute to the favourable properties of the newer agents. For example, rilmenidine lacks the histamine (H_2), receptor agonist activity of clonidine (Li *et al.*, 1989).

Most of the evidence supporting an I_1 receptor contribution to the antihypertensive action of these drugs has come from studies using local administration to the RVL. The results presented here with clonidine and guanabenz would suggest that studies examining the mechanism by which these drugs lower blood pressure when systemically administered be performed before assuming that I_1 receptor selectivity is the only factor contributing to their novel clinical profile.

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