Alteration of Contractile Function and Calcium Ion Movements in Vascular Smooth Muscle by Gentamicin and Other Aminoglycoside Antibiotics

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Experiments were conducted to examine the effects of certain aminoglycoside antibiotics on contractile responses and related calcium ion (Ca^{2+}) movements in isolated vascular smooth muscle. Gentamicin, kanamycin, and streptomycin decreased contractile responses produced by norepinephrine, histamine, and high K^+ in rabbit aortic strips. The inhibitory action of these antibiotics on mechanical function was more pronounced when the Ca^{2+} concentration of the bathing solution was decreased from 1.5 mM (normal Ca^{2+} solution) to 0.05 mM (low Ca^{2+} solution). The uptake of radiocalcium (^{45}Ca) into the isolated media-intimal layer of rabbit aortae was decreased in a maintained manner by each antibiotic. With gentamicin, the inhibitory effect on ^{45}Ca uptake was shown to be dependent upon the concentration of gentamicin employed and to be more evident in a 0.1 mM Ca^{2+} solution than in a normal Ca^{2+} solution. In addition, the rate of ^{45}Ca efflux from the rabbit aortic media-intimal layer was increased in a sustained manner by gentamicin, streptomycin, and kanamycin. Furthermore, contractile responses induced by high K^+ and norepinephrine in canine carotid arterial strips were inhibited by gentamicin. Present findings indicate that aminoglycoside antibiotics interfere with Ca^{2+} -linked events leading to activation of the contractile mechanism of vascular smooth muscle. These *in vitro* findings may partially explain the occurrence of *in vivo* cardiovascular depression that has occasionally been observed after the administration of chemically related antimicrobial agents.

Severe hypotensive episodes have been observed occasionally in man (3, 9, 24, 25) and repeatedly in experimental animals (3, 4, 18, 20, 21) after the administration of aminoglycoside antibiotics (neomycin-streptomycin group). Although the mechanism(s) involved in this adverse side effect has not been completely resolved, recent studies suggest that these agents may alter peripheral vascular smooth muscle contractility. In the constant flow-rate perfused hind-limb preparation of the dog, Cohen et al. (3) observed that the intraarterial administration of streptomycin and kanamycin decreased hind-limb vascular resistance. Evidence of streptomycin-induced dilatation of the renal vasculature was obtained in the *in situ* perfused canine kidney by Wolf and Wigton (30). The pharmacologic action involved in this peripheral vasomotor effect has not been determined, but several reports have associated this group of antibiotics with alteration of calcium ion (Ca^{2+}) function in certain mammalian organ systems.

Antagonistic interaction between the aminoglycoside antibiotics and Ca^{2+} has been recognized in the myocardium (28), autonomic ganglia (4, 5), somatic neuromuscular junction (8, 22, 23), and the perfused kidney (30).

Despite extensive documentation of the dependence of arterial smooth muscle upon Ca^{2+} for mechanical activity, the interaction of an aminoglycoside antibiotic with this cation has only recently been examined in a vascular smooth muscle system (1, 13). Neomycin, a prototypical aminoglycoside antibiotic, decreased the total uptake of radiolabeled Ca^{2+} (^{45}Ca) and induced an increase in the rate of ^{45}Ca efflux in a sustained manner in rabbit aortic strips (1). This alteration of Ca^{2+} movement was attributed to a neomycin-induced inhibition of the binding and/or the retention of Ca^{2+} at superficial sites in the arterial smooth muscle cell membrane. In this manner, neomycin markedly depressed tension responses elicited by several vasoactive agents (norepineph-

rine, serotonin, angiotensin II, histamine, and a high concentration of potassium chloride) (13). Since unanticipated alteration of vascular reactivity would be an obvious severe complication of antibiotic chemotherapy, present experiments were conducted to determine if other aminoglycoside antibiotics also altered Ca^{2+} movements and Ca^{2+} -dependent contractile responses in isolated vascular smooth muscle preparations.

MATERIALS AND METHODS

Techniques used in preparing vascular smooth muscle for *in vitro* studies have been described in detail elsewhere (1, 11-14). In the present investigation, smooth muscle strips were prepared from rabbit thoracic aortae and dog carotid arteries. Albino rabbits (New Zealand) of either sex, weighing 1.5 to 2 kg, were euthanatized by injecting a bolus of air into a marginal ear vein; the thoracic aorta was removed and placed in oxygenated solution. Mongrel dogs of either sex, weighing 15 to 25 kg, were euthanatized by rapid intravenous injection of an overdosage of pentobarbital; the external carotid arteries were removed and placed in oxygenated solution. The rabbit aorta or the canine carotid artery was cleaned and cut into spiral strips in a manner similar to that described by Furchgott (10). Intact vascular strips (comprised of adventitial, medial, and intimal layers) were used for tension studies, whereas only the media-intimal layer of the rabbit aortae, obtained by careful removal of the adventitia (19), was used in those experiments employing ^{45}Ca .

After dissection, each muscle strip was tied at both ends, attached to a stainless-steel rod under approximately 2 g of tension, and allowed to equilibrate in the appropriate bathing solution for at least 1 h prior to initiation of an experiment. The bathing medium used in this investigation was identical to the tris(hydroxymethyl)aminomethane (Tris)-buffered solution employed in previous vascular smooth muscle studies (11-13) and contained NaCl (154 mM), KCl (5.4 mM), CaCl_2 (1.5 mM), glucose (11 mM), and Tris (6 mM). Other solutions were also prepared which were similar to the Tris-buffered bathing solution except that the CaCl_2 was omitted or was reduced to either 0.1 mM Ca^{2+} or 0.05 mM Ca^{2+} . All solutions were prepared in demineralized water, adjusted to pH 7.4 with 6 N HCl, aerated with 100% oxygen, and maintained during an experiment at $34.0 \pm 0.5^\circ\text{C}$. Agents were pipetted from concentrated stock solutions directly into the bathing media to give the final concentration desired (expressed as active base). The drugs studied were potassium chloride (K^+), 1-norepinephrine bitartrate (NE), histamine dihydrochloride (Hist), and the sulfate salts of streptomycin, kanamycin, and gentamicin. The pH of the stock solutions was adjusted back to 7.4 when necessary.

Contraction of vascular smooth muscle strips was measured as isometric tension on a Grass model 7C recorder with FT.03 force displacement transducers. Smooth muscle strips were placed in tissue baths

containing 30 ml of oxygenated media and maintained under approximately 2 g of resting tension throughout an experiment. Control and experimental tension responses elicited by stimulatory agents were always obtained in each strip. The maximum heights (grams of tension) of both control and experimental contractions were measured for each muscle, and the values obtained were expressed as a percentage of the control contraction.

Calcium ion movements were estimated by measuring ^{45}Ca tissue spaces (^{45}Ca uptake) and ^{45}Ca washout (^{45}Ca efflux) in media-intimal preparations of rabbit aortae as described previously (11-13). To obtain ^{45}Ca tissue spaces, aortic strips were incubated in Tris solution for 1 h and then placed in either Tris solution or a 0.1 mM Ca^{2+} Tris solution for an additional 30 min. The muscles were then transferred to similar solutions with added ^{45}Ca (10 μM Ca^{2+} ; specific activity 0.4 $\mu\text{Ci}/\text{ml}$). After incubation in the radioactive solution for the desired time interval, tissues were removed, blotted gently with Whatman no. 5 filter paper, dipped rapidly into four successive tubes containing ^{45}Ca -free media similar to the incubation solution, and reblotted. Tissues were then weighed on a Federal-Pacific torsion balance, placed in quartz crucibles, and ashed overnight in a muffle furnace at 500°C . The ash was dissolved in 4 ml of 0.1 N HCl, and samples of this solution were placed into planchets, dried, and then counted in Nuclear-Chicago proportional gas-flow counters. The ^{45}Ca tissue space value (a tissue-media ratio expressed in terms of milliliters per gram of tissue) is obtained by dividing the amount of ^{45}Ca taken up per gram of tissue (wet weight) by the concentration of ^{45}Ca per milliliter of incubation solution. Values for ^{45}Ca uptake (micromoles per gram of muscle wet weight) may be calculated by multiplying the ^{45}Ca space values by the total concentration of Ca^{2+} (micromoles per milliliter) in the radioactive incubation media. (The total Ca^{2+} concentration is calculated as the sum of the amount of CaCl_2 added to the media, the amount of ^{45}Ca plus carrier Ca^{2+} , and the estimated amount of trace Ca^{2+} in other solution constituents. These concentrations were a maximum of 1.51 mM in the Tris solution and 0.11 mM in the 0.1 mM Ca^{2+} Tris solution.)

Aortic strips used in washout experiments were treated in the same manner as in uptake studies with the exception that (after equilibration, incubation with ^{45}Ca , dipping, and blotting) the strips were placed into successive tubes each of which contained 2 ml of nonradioactive O- Ca^{2+} solution. These washout samples were collected at 1-min intervals for 25 min, and each sample was poured into a planchet and evaporated prior to counting. At the end of the washout, the strips were then blotted, weighed, ashed, and counted. Data was expressed as the percentage of radioactivity remaining in the muscle at each 1-min interval and plotted as desaturation curves (2, 29). Also, rate constants (min^{-1}) were calculated from the slow washout component half-time ($t_{1/2}$) of the ^{45}Ca desaturation curves (0.693 divided by $t_{1/2}$) as previously described (14).

RESULTS

Preliminary experiments indicated that each of the aminoglycoside antibiotics studied (gentamicin, kanamycin, and streptomycin) depressed tension responses produced by several different vasoactive agents in aortic strips. In subsequent studies, concentrations of antibiotics which were approximately equipotent in depressing tension responses elicited by a standard dose of NE were used in both tension experiments and in studies employing ^{45}Ca .

Tension studies were conducted to characterize the inhibitory effects of 5 mM gentamicin, 10 mM kanamycin, and 10 mM streptomycin on contractile activity of aortic smooth muscle. To determine if the inhibitory actions of these antibiotics are Ca^{2+} dependent, tension responses elicited in aortic strips with NE (0.6 μM), Hist (9.0 μgM), and K^+ (80 mM) were determined in bathing media containing either a low Ca^{2+} (0.05 mM) or a normal Ca^{2+} (1.5 mM) concentration. Control tension responses of 5-min duration were obtained for each stimulatory agent in the appropriate Ca^{2+} solution; the muscle preparations were then rinsed and rested for at least 30 min. Subsequently, the strips were exposed to an antibiotic for both a 5-min period prior to addition of a stimulatory agent and a 5-min period of reexposure to NE, Hist, and K^+ . In each case, the maximum tension response elicited with a stimulatory agent was depressed by prior exposure of the aortic strip to an aminoglycoside antibiotic (Fig. 1). This inhibitory effect was of greater

magnitude in the low Ca^{2+} Tris solution than in the solution containing 1.5 mM Ca^{2+} (Fig. 1). The inhibitory effect of the antibiotics was reversed after rinsing the tissue with Tris solution.

The effects of 5 mM gentamicin, 10 mM kanamycin, and 10 mM streptomycin on total ^{45}Ca uptake were also measured. Muscle strips were incubated in bathing solution containing ^{45}Ca plus one of the three antibiotics. As can be seen in Table 1, the uptake of ^{45}Ca in the media-intimal layer of rabbit aortic strips is inhibited when an aminoglycoside antibiotic is present in the incubation medium. To determine whether this effect was due to an alteration of the rate of Ca^{2+} uptake rather than an inhibition of the uptake of a specific fraction of Ca^{2+} , ^{45}Ca tissue spaces were obtained by incubating aortic strips for several different time intervals in a 0.1 mM Ca^{2+} bathing solution containing ^{45}Ca and gentamicin. The decrease in ^{45}Ca spaces induced by gentamicin in this low Ca^{2+} solution was constant between the 40- and 90-min incubation periods (Table 2). Measurements with kanamycin and streptomycin indicated a similar maintained inhibitory effect on ^{45}Ca uptake.

A previous study had indicated that the inhibitory effect of neomycin on NE-induced tension responses was directly related to the concentration of antibiotic employed (13). In the same study, the inhibitory effect of neomycin on contractile responses of aortic strips was demonstrated to be inversely related to the Ca^{2+} concentration of the bathing medium. In the present investigation, different concentrations of gentamicin were used in ^{45}Ca uptake experiments to determine if the inhibitory effects of gentamicin on Ca^{2+} uptake were also dose dependent and Ca^{2+} dependent. As can be seen in Fig. 2, inhibition of ^{45}Ca uptake by gentamicin is clearly dose dependent over a wide range of concentrations of the antibiotic. In addition, inhibition of ^{45}Ca uptake by gentamicin is significant at lower concentrations of the antibiotic in the medium containing the lower (0.1 mM) Ca^{2+} concentration (Fig. 2).

Washout experiments with ^{45}Ca indicated that neomycin produced a sustained increase in ^{45}Ca efflux from rabbit aortic strips; this increase persisted after prior incubation of the tissue with strontium (which penetrates the cell membrane), but was absent when ethylenediaminetetraacetic acid (EDTA) (which does not penetrate the cell membrane) was also present (13). These findings were

FIG. 1. Inhibition by aminoglycoside antibiotics of isometric contractions produced by NE (0.6 μM ; ●), histamine (9.0 μM ; Δ) or K^+ (80 mM; ○) in rabbit aortic smooth muscle strips. Gentamicin (5 mM), streptomycin (10 mM), or kanamycin (10 mM) was added 5 min prior to and during the 5-min exposure to a stimulatory agent. Each value is the mean $\pm$ SE of three to nine responses. Inhibition of responses by antibiotics was significantly greater ($P < 0.05$ to $P < 0.001$) in the lower Ca^{2+} concentration except with the two values designated by * ($P > 0.05$).

TABLE 1. *Effects of aminoglycoside antibiotics on ^{45}Ca tissue spaces in 1.5 mM Ca^{2+} in the media-intima of rabbit aortic strips^a*

Antibiotic	^{45}Ca space (ml/g $\pm$ SE)	^{45}Ca space difference from controls ^b (ml/g $\pm$ SE)	Significance ^c
Control	1.87 $\pm$ 0.036		
Gentamicin (5 mM)	1.58 $\pm$ 0.063	0.29 $\pm$ 0.066	$P < 0.005$
Streptomycin (10 mM)	1.47 $\pm$ 0.025	0.40 $\pm$ 0.043	$P < 0.0005$
Kanamycin (10 mM)	1.47 $\pm$ 0.091	0.40 $\pm$ 0.098	$P < 0.005$

^a Values are mean $\pm$ SE of four paired experiments. Strips were incubated with an antibiotic for 10 min prior to exposure to ^{45}Ca and during the 20-min period that ^{45}Ca was present.

^b Mean differences $\pm$ SE between values for paired control and antibiotic-treated strips.

^c Student's paired t test for control and antibiotic-treated muscles.

TABLE 2. *Effects of gentamicin on ^{45}Ca tissue spaces in 0.1 mM Ca^{2+} in the media-intima of rabbit aortic strips^a*

Incubation interval (min)	Control	Gentamicin (ml/g $\pm$ SE)	Difference
20	6.979 $\pm$ 0.592	6.683 $\pm$ 0.504	0.2960 $\pm$ 0.1621 ^b
40	8.153 $\pm$ 0.317	6.953 $\pm$ 0.631	1.1998 $\pm$ 0.6320 ^b
60	9.051 $\pm$ 0.689	7.893 $\pm$ 0.488	1.1582 $\pm$ 0.4891 ^b
90	9.677 $\pm$ 0.674	8.725 $\pm$ 0.445	0.9527 $\pm$ 0.3725 ^b

^a Values are mean $\pm$ SE of six paired experiments. Treated strips were incubated with gentamicin for 10 min prior to and during the incubation period.

^b Gentamicin value significantly less ($P < 0.05$) than corresponding control value.

interpreted to indicate that neomycin affected ^{45}Ca efflux by interfering with the reuptake or rebinding of a fraction of Ca^{2+} located at superficial membrane sites. Washout experiments with ^{45}Ca were performed in the present investigation to determine if other aminoglycoside antibiotics also altered Ca^{2+} efflux in a similar manner. After incubation of aortic strips in Tris bathing solution containing ^{45}Ca , muscles were washed out in successive tubes containing nonradioactive O-Ca^{2+} solution for 25 min. During the last 10 min of the 25-min washout, an antibiotic was also present in the O-Ca^{2+} solution. As illustrated in Fig. 3, a pronounced maintained increase in ^{45}Ca efflux is obtained when the tissue is exposed to an aminoglycoside antibiotic. Rate constant values calculated from these data are summarized in Table 3 and indicate the magnitude of the increase in net ^{45}Ca efflux rate obtained after exposure of aortic strips to each of the antibiotics.

The experiments with rabbit aortic strips show that aminoglycoside antibiotics modify tension responses of vascular smooth muscle (Fig. 1). Similar experiments were conducted with dog carotid strips for the purpose of

determining whether gentamicin also depressed contractile activity of a more peripheral vascular smooth muscle in another mammalian species. Control tension measurements were obtained with NE and K^+ in dog carotid strips for a 5-min period. After resting the tissues for at least 30 min, tension responses were reobtained after a 5-min exposure to 5 mM gentamicin in the same manner as in rabbit aortic strips. In a total of eight experiments with tissues obtained from four dogs, gentamicin depressed contractile responses elicited with NE and K^+ by $36.5 \pm 0.7\%$ and $33.1 \pm 3.2\%$ (mean $\pm$ standard error [SE]), respectively. Typical examples of gentamicin-induced depression of tension responses of dog carotid strips are illustrated in Fig. 4.

A preliminary report indicated that neomycin produced a very slight contractile response in rabbit aortic strips in the absence of any vasoconstrictor agent (1). However, in the present study with several aminoglycoside antibiotics and in an extensive study with neomycin and various canine arteries (H. R. Adams, F. R. Goodman, and G. B. Weiss, Fed. Proc. 33:567, 1974), these antibiotics did not appear to alter the resting tone of noncontracted vascular smooth muscle strips.

FIG. 2. Effect of different concentrations of gentamicin on ^{45}Ca tissue spaces in 0.1 mM Ca^{2+} and 1.5 mM Ca^{2+} in media-intimal layer of rabbit aortic strips. Each value is the mean $\pm$ SE of six to 12 experiments. Treated strips were incubated with gentamicin for 10 min prior to and during the 20-min incubation with ^{45}Ca . Values designated with *, **, ***, are significantly less ($P < 0.05$, $P < 0.005$, $P < 0.0005$, respectively) than corresponding control value.

DISCUSSION

Depression of cardiovascular function by antimicrobial agents has been the subject of several controlled laboratory studies involving nonhuman animals (3, 18, 20, 21, 28). Apparently, this adverse drug response occurs infrequently during therapeutic situations in man (3, 28); however, occasional clinical reports have associated the aminoglycoside group of antibiotics with unusual hemodynamic responses. Ream (25), for example, documented an incidence of profound hypotension and acute cardiac arrest occurring immediately after the intravenous administration of kanamycin in a patient with an appendical abscess. Depression of systemic blood pressure in man has also been observed by several investigators after the administration of other chemically related antibiotics (3, 9, 24). Although several mechanisms (e.g., negative inotropic and chronotropic effects on the myocardium [3, 18, 28], autonomic ganglionic blockade [4, 5, 21], and respiratory depression [21, 25]) have been proposed as potential mediating factors of antibiotic-induced cardiovascular effects, present findings

indicate that direct inhibition of vascular smooth muscle contractility may be involved in the etiology of the hemodynamic depressant actions of the aminoglycoside antibiotics.

Contractile responses produced by NE, Hist, and high K^+ in rabbit aortic smooth muscle were significantly reduced by exposure of the tissue to gentamicin, kanamycin, and streptomycin. The demonstration that responses to NE and high K^+ were also decreased by gentamicin in smooth muscle strips prepared from dog carotid arteries indicates that the inhibitory actions of aminoglycoside antibiotics may occur in a variety of vascular tissues in different mammalian species. Also, previous studies have indicated that vascular smooth muscle contractile responses elicited with angiotensin II and serotonin (as well as with NE, Hist, and high K^+) were inhibited by neomycin (13), another aminoglycoside antibiotic. These antibiotics, therefore, alter contractile responses that are mediated by several different pharmacologic agents. The points of action of these diverse agents, include activation of adrenergic, histaminergic, and serotonergic receptor sites,

FIG. 3. Effects of aminoglycoside antibiotics on ^{45}Ca efflux in media-intima of rabbit aortic strips. Each curve represents a summary of four separate experiments. Strips were incubated with added ^{45}Ca for 2 h prior to washout in an O-Ca Tris solution. Arrows indicate the addition to the washout solutions of either streptomycin (B), gentamicin (C), or kanamycin (D). Dashed lines represent extrapolation of the washout curves prior to addition of an antibiotic.

TABLE 3. Alteration of rate constant of slow component of ^{45}Ca washout by addition of antibiotics^a

Antibiotic added during washout	Slow component rate constant (min^{-1})		Increase in rate constant (min^{-1})	P
	Before antibiotic	After antibiotic		
Control	0.026 ± 0.002	0.026 ± 0.002		
Gentamicin (5 mM)	0.024 ± 0.002	0.035 ± 0.007	0.011 ± 0.006	<0.05
Streptomycin (10 mM)	0.023 ± 0.001	0.050 ± 0.005	0.027 ± 0.005	<0.005
Kanamycin (10 mM)	0.021 ± 0.002	0.076 ± 0.013	0.055 ± 0.013	<0.005

^a Each value represents the average $\pm$ SE of values from four paired experiments. P values were calculated for the average of the differences between paired muscles.

FIG. 4. Inhibition by gentamicin of NE- and K^+ -induced contractions of smooth muscle strips prepared from canine carotid arteries. Gentamicin (5 mM) was added 5 min prior to and during the addition of the stimulatory agent.

as well as a direct depolarizing action on the smooth muscle cell membrane. Thus, it would appear that suppression of vascular smooth muscle contractility by aminoglycoside antibiotics involves a rather nonspecific inhibitory mechanism.

It has been thoroughly documented that Ca^{2+} is essential for activation of the contractile mechanism of a wide variety of smooth muscle systems (7, 15, 17, 26, 27). In vascular smooth muscle, the way in which Ca^{2+} is taken up, distributed, bound to cellular constituents, and subsequently utilized may play an important role in determining the manner in which the tissue responds mechanically to vasoactive agents (26, 27). In the present study, tension responses produced by high K^+ , Hist, and NE were depressed by gentamicin, kanamycin, and streptomycin to a greater extent when the smooth muscle was immersed in a low Ca^{2+} solution. These findings suggest that the aminoglycoside antibiotics alter vascular smooth muscle contractility by interfering with the disposition and/or utilization of Ca^{2+} by the smooth muscle fiber.

Gentamicin, streptomycin, and kanamycin produce specific alterations in ^{45}Ca movements as well as a Ca^{2+} -related inhibition of vascular

smooth muscle contractility. The uptake of ^{45}Ca by the media-intima of rabbit aortae was decreased by exposure of the tissue to these antibiotic agents. As demonstrated with gentamicin, this inhibitory action was dependent upon the concentration of antibiotic employed and was more pronounced when the tissue was incubated in a low Ca^{2+} medium. In addition, the inhibitory effect of these antibiotics on ^{45}Ca uptake was maintained over all incubation time intervals studied. Thus, as previously suggested for neomycin (13), aminoglycoside antibiotics do not simply decrease the rate of ^{45}Ca influx, but they appear to interfere with the uptake of a specific fraction of Ca^{2+} .

Washout studies with ^{45}Ca also suggested an effect of the aminoglycoside antibiotics on a specific portion of cellular Ca^{2+} . The net efflux rate of ^{45}Ca was markedly increased by exposure of the aortic strips to gentamicin, kanamycin, and streptomycin. With neomycin, this effect has been shown to persist after incubation of the tissue with strontium but not after exposure of the strips to EDTA (13). Strontium penetrates the smooth muscle cell membrane and binds at a proportion of Ca^{2+} -susceptible sites (6, 16, 17), whereas EDTA increases the apparent rate of ^{45}Ca washout by decreasing the concentration of extracellular Ca^{2+} that would otherwise be available for reuptake into the cell membrane (2, 6, 12, 14). It was suggested, therefore, that neomycin and perhaps other chemically related antibiotics affected the uptake and/or retention of Ca^{2+} by decreasing the availability or affinity of superficial Ca^{2+} binding sites in the vascular smooth muscle cell membrane (1, 13). The present observation that gentamicin, streptomycin, and kanamycin produce a similar sustained increase in ^{45}Ca efflux supports this contention. By affecting this Ca^{2+} fraction, the aminoglycoside antibiotics may alter the ability of vascular smooth muscle to react to endogenous and/or exogenous stimuli that normally

utilize this as well as other Ca^{2+} -linked components to initiate and maintain vascular contractile responses.

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