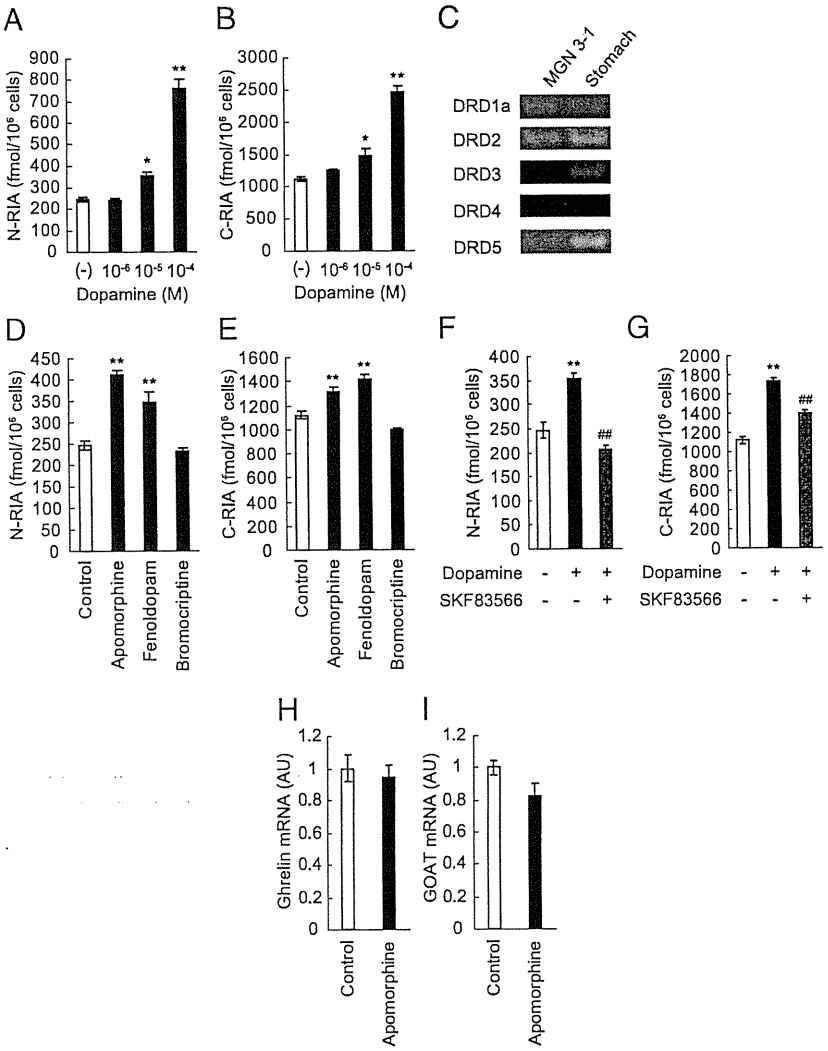

FIG. 5. The effect of dopamine on ghrelin secretion by MGN3-1 cells. A and B, The amount of ghrelin secreted by MGN3-1 cells incubated for 4 h in DMEM supplemented with 0.5% BSA and 10^{-6} to 10^{-4} M of dopamine. *, $P < 0.05$, **, $P < 0.01$ in comparison with controls (−) (n = 9). C, RT-PCR analysis of dopamine receptor (DR) D1a and D2–5 mRNA expression in MGN3-1 cells. D and E, The amount of ghrelin secreted by MGN3-1 cells incubated for 4 h in DMEM supplemented with 0.5% BSA and 10^{-5} M apomorphine (nonselective dopamine agonist), fenoldopam (D1 agonist), or bromocriptine (D2, D3 agonist). **, $P < 0.01$ in comparison with controls (n = 9). F and G, The amount of ghrelin secreted by MGN3-1 cells incubated for 4 h in DMEM supplemented with 0.5% BSA and 10^{-4} M dopamine with or without 10^{-4} M SKF83566 (D1 antagonist). **, $P < 0.01$ in comparison with controls; ##, $P < 0.01$ in comparison with dopamine (n = 9). H and I, Ghrelin and GOAT mRNA levels in MGN3-1 cells after a 24-h incubation with 10^{-5} M apomorphine (n = 9). AU, Arbitrary unit.

which in turn influences various homeostatic systems, including energy homeostasis or growth control. We sought to understand better the molecular mechanisms governing ghrelin secretion by cells, which may further contribute to understanding the physiological role of ghrelin. In previous studies, we have developed a ghrelin-secreting cell line MGN3-1 as a research tool to study the regulation of ghrelin secretion *in vitro* (12). In this study, we examined the effects of the various peptide hormones and neurotransmitters on ghrelin secretion using MGN3-1 cells.

We found that oxytocin significantly stimulates ghrelin secretion from MGN3-1 cells. Oxytocin, a nonapeptide with a disulfide bond, is secreted from the posterior pituitary gland in a neuroendocrine manner and is involved in milk ejection and uterine contraction. Oxytocin also acts as a neurotransmitter, specifically as a negative regulator of food intake to oxytocin-receptive neurons in the paraventricular nucleus of the hypothalamus (19). Only two previous reports have examined the effect of oxytocin on plasma ghrelin levels. Vila *et al.* (20)described a reduction in basal and lipopolysaccharide-induced ghrelin levels in healthy men after systemic administration of oxytocin. Shibata *et al.* (21) reported that inhibition of the suckling-induced increase in plasma oxytocin levels by a oxytocin antagonist did not alter plasma ghrelin levels in lactating rats. Although the investigators concluded that oxytocin has no effects on ghrelin secretion, our findings are not in accordance with that report. The reason for this discrepancy is not clear but may result from indirect effects of additional mediators *in vivo*. Further studies will be needed to explore the regulation of ghrelin secretion by oxytocin *in vivo*.

We also found that the nonpeptide neurotransmitters epinephrine and norepinephrine strongly stimulate ghrelin secretion by MGN3-1 cells. Ghrelin secretion has been suggested to be regulated by the sympathetic nervous system. Mundinger *et al.* (22) noted that increased portal ghrelin levels in rats after electrical sympathetic nerve stimulation or iv tyramine administration. Hosoda and Kangawa (23) reported that the administration of adrenergic agonists increased plasma ghrelin levels in rat. Recently Zhao *et al.* (24) reported that ghrelin secretion from the pancreatic ghrelinoma cell line PG-1 and the stomach ghrelinoma cell line SG-1 could be stimulated by β1-adrenergic receptors. Our observation demonstrating increased ghrelin secretion after epinephrine and norepinephrine administration is consistent with these results, supporting the idea that sympathetic nervous system is an important regulator of ghrelin secretion.

In addition to epinephrine and norepinephrine, dopamine also significantly stimulated ghrelin secretion from MGN3-1 cells via the D1A receptor. As far as we know, this is the first report of ghrelin secretion stimulation by dopamine. Dopamine is a catecholamine, acting as a neurotransmitter in the certain brain areas in motor control or reward behaviors. A substantial amount of dopamine is also produced in the gastrointestinal tract (25), in which it suppresses gastric motility, stimulates exocrine secretions, modulates jejunal sodium absorption, or protects against gastroduodenal ulcers (26, 27). Our finding raises the possibility that gastrointestinal dopamine may also control ghrelin secretion.

In this study, we used a standard culture medium (DMEM) for the incubation study. The medium contains several compounds including inorganic salts, glucose, amino acids, or vitamins, the concentrations of which may not be entirely the same to that around the ghrelin cell *in vivo*. We cannot exclude the possibility that these compounds may have influenced on the results and that may explain the discrepancy between our data and clinical studies of oxytocin. Further studies will be needed to clarify the combinational effects of these compounds in the medium and peptide hormones or neurotransmitters.

In addition to epinephrine and norepinephrine, which were previously known to increase ghrelin secretion, we identified two new regulators of ghrelin secretion, oxytocin and dopamine, by screening peptide hormones and neurotransmitters using MGN3-1 cells. These findings will provide new direction for further studies seeking to understand better the regulation of ghrelin secretion and the overall physiological role of ghrelin in organism homeostasis and energy regulation.

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