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EFFECTS OF CALCIUM CHANNEL ACTIVATORS BAY-K8644 AND CGP-28392 ON STEROIDOGENESIS IN GRANULOSA CELLS OF THE DOMESTIC HEN

Elikplimi K. Asem and Benjamin K. Tsang

Reproductive Biology Unit, Department of Obstetrics and Gynecology and Physiology, University of Ottawa, Ottawa Civic Hospital, Ottawa, Ontario, Canada K1Y 4E9

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Summary: The effects of calcium agonists BAY-K8644 and CGP-28392 on steroidogenesis was examined in chicken granulosa cells in short term incubation. BAY-K8644 (5-500 nM) and low doses of CGP-28392 (1-10 μM) failed to appreciably affect basal and LH-stimulated progesterone production whether tested in calcium free, low (0.05 mM) or high (3 mM) calcium containing medium. However, higher concentrations of CGP-28392 (50-250 μM) inhibited significantly ($P < 0.01$) both basal and LH-stimulated steroidogenesis in a dose-related manner independently of extracellular calcium availability. The suppressive effect of CGP-28392 was manifest with submaximally and maximally stimulating LH doses. In additional experiments with non-hormonal agonists such as forskolin, dibutyryl cyclic AMP and kaurenol, BAY-K8644 and low CGP-28392 concentrations were again without effect on steroidogenesis. By comparison, higher CGP-28392 doses suppressed the stimulatory effects of all three agonists dose-dependently. These results demonstrate that, the calcium channel agonists are incapable of inducing a steroidogenic response in chicken granulosa cells. Since BAY-K8644 and CGP-28392 (low dose, 1-10 μM) failed to influence steroidogenesis in the dose range that induced maximal physiologic responses and calcium influx in a variety of cells, it is concluded that chicken granulosa cells lack the type(s) of channels specific for them. Hence the usefulness of BAY-K8644 and CGP-28392 as Ca^{2+} probes may be tissue-specific. The inhibitory effects of CGP-28392 appear to be non-specific.

The presence of calcium ions in the incubation medium is vital for the full expression of gonadotropin-stimulated steroidogenesis in rat (1), swine (2) and chicken (3) granulosa cells. Furthermore the steroidogenic actions of non-hormonal agonists

The Abbreviations used are:- LH, luteinizing hormone; BAY, BAY-K8644; CGP, CGP-28392; Bu_2cAMP, dibutyryl adenosine 3'-5' cyclic monophosphate.

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such as cholera toxin, forskolin and dibutyryl cyclic AMP require the presence of extracellular calcium (1-3).

However, the relative contribution of extracellular calcium in agonist-induced progesterone synthesis has not been clearly defined. In the present study, we have examined the effects of two closely related dihydropyridine calcium channel activators, BAY-K8644 (BAY) and CGP-28392 (CGP), as potential probes on acutely stimulated steroidogenesis in chicken granulosa cells. BAY-K8644 is structurally related to the calcium channel antagonist nifedipine and has been shown to increase calcium influx and enhance contraction in smooth and heart muscle (4, 5). CGP is also related to nifedipine and is capable of stimulating calcium influx into platelets (6, 7) and heart cells (8).

MATERIALS AND METHODS

BAY was donated by Miles Laboratory, New Haven CT; and CGP was a gift from Ciba-Geigy Co., Summit. N.J., and Kaurenol also a gift from Dr. K. Fuji, Kyoto University, Kyoto, Japan. Other hormones and chemicals used in this study have been described (1).

One-year-old white leghorn hens were generously provided by the Animal Research Centre, Agriculture Canada, Ottawa, Canada and were housed individually in windowless environmental-controlled rooms and had free access to water and a layer ration under 14L:10D (light:dark) cycle. Granulosa cells pooled from the largest and the second largest preovulatory follicles (F_1 and F_2) 16-18 h before ovulation of the F_1 were incubated (3) either in Ca^{2+} free Eagles Minimum Essential medium with Earle's salts (MEM) or in Medium 199 (M199) containing Hank's salts (Ca^{2+}, 1.3 mM), at 37^{0}C for 3 h. All media were buffered with 25 mM hepes (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) pH 7.4. Total progesterone (medium + cells) was measured by radioimmunoassay (1).

Results presented are mean $\pm$ SEM of three separate experiments each performed in triplicates with granulosa cells pooled from F_1 and F_2 of two hens. The data were subjected to analyses of variance and post-hoc Tukey's test.

RESULTS

LH-stimulated progesterone synthesis increased steadily with increasing concentrations of Ca^{2+} in the external medium, reaching a maximum at 1 mM Ca^{2+}. BAY (5-100 nM) did not have

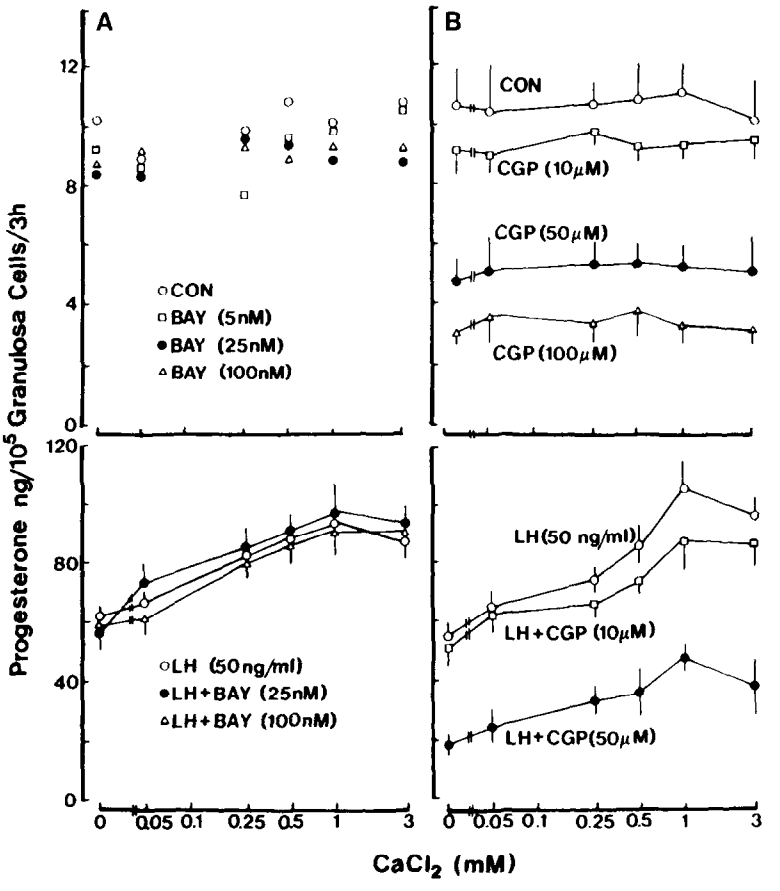

Fig. 1. Effects of BAY (A) and CGP (B) on basal and LH-stimulated progesterone production in Ca^{2+} free, low and high Ca^{2+} containing MEM. Each point is mean ± SEM of three separate experiments each performed in triplicates.

any appreciable effect on unstimulated (basal) and LH-stimulated steroidogenesis whether tested in calcium free, low (0.05 mM) or high (3 mM) calcium containing medium (Fig. 1A). Whereas CGP at concentrations between 1 and 10 μM had no effect on the basal and LH-induced steroidogenic response both in the presence and absence of extracellular calcium, a larger dose of 50 μM was inhibitory (Fig. 1B). Potassium concentration of the incubation media was 5 mM. In the next series of experiments the extracellular calcium concentration was maintained at 1.3 mM and the concentrations of LH and calcium agents varied. Fig. 2A shows that BAY in the concentration range of 5-500 nM also failed to

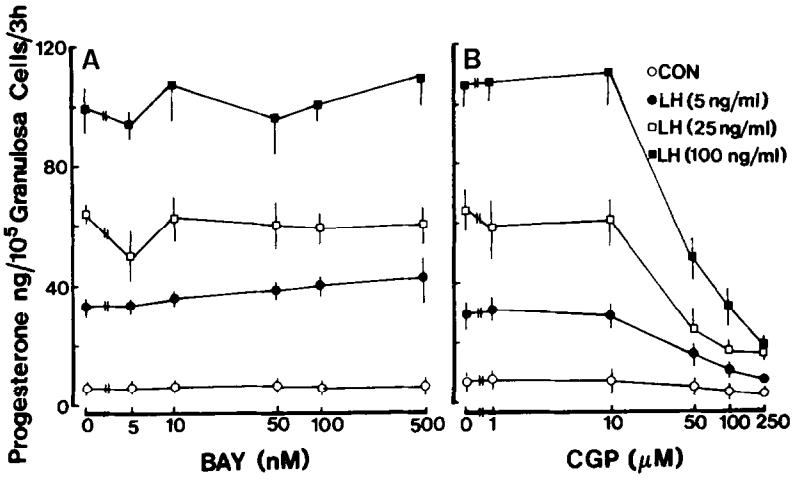

Fig. 2. Effects of low and high doses of BAY (A) and CGP (B) on basal and LH-stimulated progesterone production in M199 (Ca^{2+} = 1.3 mM). Values are mean ± SEM from three experiments performed in triplicates.

influence the actions of submaximally and maximally stimulating LH concentrations. Under identical conditions low dose of CGP (1-10 μM) was without effect although higher concentrations (50-250 μM) significantly ($P < 0.05$) suppressed both basal and LH-stimulated activities in a dose-dependent manner (Fig. 2B).

To determine if the inhibitory action of CGP was due to suppression of a cyclic AMP-dependent event, we incubated granulosa cells with forskolin, Bu_2cAMP and kaurenol in the presence of varying concentrations of the calcium agonists in M199 containing 1.3 mM calcium. All three agonist stimulated progesterone production in a dose-related fashion (Fig. 3). Whereas, BAY (Fig. 3B) and low CGP levels (Fig. 3B) were again without effect, higher CGP doses attenuated the steroidogenic actions of the three non-hormonal agonists (Fig. 3B).

DISCUSSION

Results from the present study demonstrate that avian granulosa cells are insensitive to the calcium channel agonists BAY and CGP as far as steroidogenesis is concerned. This conclusion

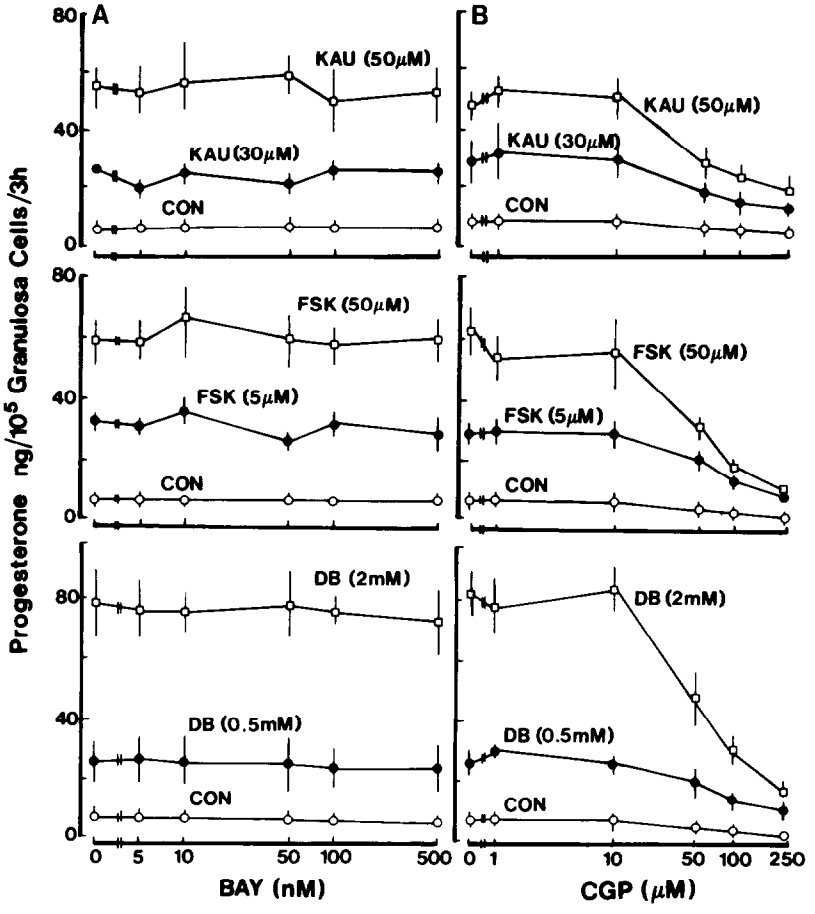

Fig. 3. Effects of low and high concentrations of BAY (A) and CGP (B) on Bu_2cAMP(DB)-, forskolin(FSK)-, kaurenol (KAU)-induced progesterone production in M199 (Ca^{2+} = 1.3 mM). Results are mean ± SEM from three experiments done in triplicates.

stemmed from the observation that these agonists, at concentrations effective in eliciting a variety of physiologic responses in various cell types (4, 5, 7), failed to enhance basal, gonadotropin and non-hormonal agonist-stimulated progesterone production. Furthermore, BAY and low concentrations of CGP had no effect on the steroidogenic actions of forskolin and Bu_2cAMP, both of which are known to raise intracellular cAMP levels although by different mechanisms and of kaurenol which stimulates adrenal steroidogenesis without increasing cAMP production (9).

It is noteworthy that in the present study, the extracellular calcium accounted for only 45-50% of LH-induced steroidogenesis confirming previous results (3). It is possible that calcium mobilized from intracellular source(s) is responsible, at least in part, for the significant agonist-induced progesterone response in avian granulosa cells (10). Although it is well established that extracellular calcium plays a significant role in the regulation of steroidogenesis in granulosa cells (1-3) it is not known how such calcium is made available for steroid biosynthetic processes. Our present observations suggest that BAY and CGP are not useful probes for clarification of the role of extracellular calcium in our system. The insensitivity of avian granulosa cells may be due to the lack of specific receptors for dihydropyridine derivatives and/or the absence of such channels activated by them. Indeed, the existence of specific receptors for dihydropyridines have been demonstrated in some excitable tissues (11-13).

It is also possible that concentrations of the Ca^{2+} agonists higher than those reported in the literature might be effective in the present system. However, BAY remained without effect at higher concentrations and, CGP was unexpectedly inhibitory on the basal and LH-stimulated response independently of Ca^{2+} availability. This action of CGP, coupled with its suppressive effects on the stimulatory actions of diversely acting non-hormonal agonists, suggests that this agent may be acting non-specifically in inhibiting steps before and after cyclic AMP generation. Furthermore, it is possible that CGP may inhibit some cyclic AMP-independent steps because it was capable of attenuating the steroidogenic response to kaurenol. Non-specific and calcium-independent inhibitory actions of CGP has also been reported (4, 14).

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