EFFECT OF COLCHICINE ON STEROID SECRETION FROM RAT ADRENAL GLAND

Minoru INABA and Kunie KAMATA

Department of Pharmacology, Kyorin University School of Medicine, Shinokawa, Mitaka-shi, Tokyo 181, Japan

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Abstract—To determine the influence of colchicine on adrenocortical function in vivo, we gave significant amounts subcutaneously to rats and observed the distribution to the anterior pituitary and adrenal glands. Corticosteroids in the serum and adrenal gland were increased remarkably by the administration of colchicine in a dose-dependent manner. Maximum elevation was achieved around 2.5 hours after the injection and continued for at least 4 hours. Maximum levels produced by the injection of 1 mg/kg body wt. of colchicine were the same as those elicited by the injection of 0.1 U/rat of adrenocorticotropic hormone, the latter producing temporary increases in corticosteroids in the serum and adrenal gland. A significant augmentation of corticosteroids in the serum and adrenal gland resulted when colchicine was administered 60 minutes prior to the injection of adrenocorticotropic hormone. The pattern of intracellular distribution of corticosteroids in the adrenal glands of rats given colchicine was the same as that seen in rats given adrenocorticotropic hormone. In the in vitro experiment, only high concentrations of colchicine such as $10^{-3}$ M added to the incubation system of the adrenal quarters suppressed the release of corticosteroids from the adrenal gland. Thus, the continuous stimulation of the synthesis and secretion of adrenocortical hormone seen with the administration of colchicine may explain why this drug is effective in the treatment of gouty arthritis.

Colchicine has long been effectively used for cases in acute gouty arthritis, but the mode of action is not entirely understood. A possible relationship between the genesis of acute gouty arthritis and inflammatory reaction of the knee joint produced by the local injection of urate crystals has been suggested (1), but colchicine administration does not produce a decrease in the concentration of uric acid in the serum or promote the excretion of uric acid. One approach for clarifying the basic mechanism of colchicine action is to examine the effect of the agent on the function of adrenocortical tissue which produces steroids possessing anti-inflammatory properties. Since Lacy et al. suggested that the microtubular system in the islet cells of the pancreas probably plays an important role in the secretion of insulin from these cells (2), several in vitro experiments have been carried out on the pituitary, thyroid and adrenal glands concerning the influence of colchicine and other antimicrotubular agents on endocrine functions (3-8). A consensus of opinion, however, has never been reached, and few in vivo experiments clarifying the effect of colchicine and other antimicrotubular agents on adrenocortical function have been documented.

The present study was carried out to examine the influence of colchicine administration on adrenocortical function in rats in vivo. Direct effect of the agent on the release of corticosteroids from adrenal gland was also studied in the in vitro experiment.
MATERIALS AND METHODS

Chemicals: Colchicine, purchased from Merck, was dissolved in saline for injection or addition to the incubation system. \([\text{H}]\) colchicine (5.0 mCi/mg) was purchased from the Radiochemical Centre, Amersham, and kept refrigerated. Adrenocorticotropic hormone (ACTH) (Cortorosyn, N.V. Organon) was purchased from the Daiichi Pharmaceutical Co., Tokyo.

Animals: Male Donryu rats weighing approx. 300 g were fed on laboratory chow (CE-2, Nihon CLEA Co., Tokyo) and water ad libitum in an air-conditioned room (24 ± 1 °C) that was illuminated from 6:00 A.M. to 6:00 P.M. Pertinent care of the rats was performed by “handling” including weighing and injecting 0.1 ml of saline s.c. into tissues on their backs between 10:00 and 10:30 every morning for at least 10 days before the experiments.

Procedures for studying the distribution of colchicine to the organs: A mixture of 100 µCi/kg of \([\text{H}]\) colchicine and 1 mg/kg of unlabelled colchicine was injected s.c. into tissues on the backs of intact rats. The animals were decapitated using a guillotine-type cutter 2 hr later. Organs such as the liver, kidney, adrenal gland, anterior pituitary gland and brain were immediately removed. After weighing and homogenizing the organs or parts of the organs in 5–10 vol. of 33% ethanol using a glass-teflon homogenizer in ice-cold water, the tissue homogenates were extracted 3 times using dichloromethane. The extract was evaporated to dryness, and the dry residue was dissolved in an appropriate vol. of methanol. \(^{3}\text{H}\) radioactivity in the aliquots of methanol solution was counted in a Tri-Carb liquid scintillation spectrometer (Model 3224, Packard Instruments Co.) using 10 ml/vial of PPO-POPOP-toluene scintillator. Counting efficiency was 42% and counting error was within ±1%.

Procedures for in vivo experiments: A single dose of colchicine (0.5, 1 or 2 mg/kg body wt.) was given s.c. into tissues on the backs of rats. An equal vol. of saline was injected s.c. into the control rats. One experimental group of rats included 5 animals from the same litter. The administration of the drug was performed between 10:00 and 10:30 A.M. ACTH (0.1 U/rat) was given s.c. 60 or 120 min after colchicine administration, and the animals were decapitated 30 min after ACTH administration. In a time-course study, rats from one group given colchicine were decapitated at varying intervals of 1.5, 2.5, 4.5, and 6.5 hr after the injection. Blood from the necks of decapitated rats was collected in conical centrifuge tubes and the adrenal glands were removed immediately after the collection of blood. Corticosteroids in the serum and adrenal gland were fluorometrically determined by the slightly modified method of Guillemin et al. (9, 10). Corticosterone was used as a reference standard. Intracellular distribution of corticosteroids in the adrenal gland was studied by the method previously described (10, 11). Protein was chemically assayed by the method of Lowry et al. (12).

Procedures for in vitro experiments: Adrenal quarters of rats were incubated in Krebs-Ringer bicarbonate buffer solution containing 0.2% glucose (pH 7.4) at 37°C in a gas phase of 95% O₂-5% CO₂ according to the method of Saffran and Schally (13). Incubation of the tissues with colchicine (10⁻³–10⁻⁵ M) was carried out simultaneously under the same
conditions. As colchicine produced fluorescence and interfered with the fluorometric assay of corticosteroids in reaction with H₂SO₄, blank incubations containing only colchicine at various concentrations without the tissue were also carried out simultaneously. Values obtained from the blank incubations were subtracted from those obtained from the tissue incubations with colchicine.

RESULTS

Distribution of colchicine to the organs: Two hr after a single dose of colchicine was injected into the intact rats, the highest ³H-radioactivity per g tissue was found in the liver. The concentration of radioactivity in the kidney was also high, though it was a little less than in the liver. Radioactivity per unit wt. of tissue in the anterior pituitary and adrenal glands was noticeably high, i.e. approx. 60% of the concentration in the liver was found in the anterior pituitary gland, and 60% of the concentration in the pituitary gland was found in the adrenal gland. In contrast to the high distributions to these organs, considerably less colchicine was distributed in the cerebrum, cerebellum and hypothalamus (Table 1).

Effect of systemic administration of colchicine on corticosteroid levels in the serum and adrenal gland: A subcutaneous administration of a single dose of colchicine to intact rats produced long-lasting increases in corticosteroids in both the serum and adrenal gland. Corticosteroid levels increased continually, reaching a maximum approx. 2.5 hr after the injection. Maximum levels were maintained for at least 4 hr. The increase in corticosteroids was produced in a dose-dependent manner (Fig. 1). When ACTH was given s.c. into colchicine-pretreated rats before increases in corticosteroids due to prior injection of colchicine had reached maximum levels, a significant augmentation in these increases was obtained and the increased levels reached maximum both in the serum and in the adrenal gland (Fig. 2). Such significant augmentation could not be obtained when ACTH was injected 2 hr after colchicine, that is, at a time when levels of serum and adrenal corticosteroids had almost reached a maximum as a result of colchicine injection (Fig. 3). Intracellular distribution patterns of corticosteroids in the adrenal gland were studied using

Table 1. Distribution of colchicine to rat organs

<table>
<thead>
<tr>
<th>Organs</th>
<th>³H-radioactivity (dpm/g tissue)</th>
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<tbody>
<tr>
<td>Liver</td>
<td>130 350 ± 16 570*</td>
</tr>
<tr>
<td>Kidney</td>
<td>106 090 ± 2 990</td>
</tr>
<tr>
<td>Anterior pituitary gland</td>
<td>76 200 ± 9 435</td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>46 200 ± 3 070</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>945 ± 40</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1 150 ± 170</td>
</tr>
<tr>
<td>Hypothalamus tissue</td>
<td>1 360 ± 120</td>
</tr>
</tbody>
</table>

A mixture of unlabelled colchicine (1 mg/kg body wt.) and [³H]colchicine (100 µCi/kg body wt.) was given s.c. to the 5 intact rats. The organs were removed from the decapitated rats 2 hr after the injection. Radioactivity in the tissues was determined by the method described in Materials and Methods. *Mean ± S.D.
intact rats, colchicine-treated rats, ACTH-treated rats and colchicine-ACTH-treated rats. As seen in Fig. 4, the pattern of colchicine-treated rats was the same as in ACTH-treated and colchicine-ACTH-treated rats.

Direct effect of colchicine on release of corticosteroids from adrenal gland: Addition of colchicine to the incubation system of rat adrenal quarters resulted in decreases in corticosteroids in the incubation medium and the cytosol fraction of the incubated tissue only at high concentrations such as $10^{-3}$ and $10^{-4}$ M. Inhibition of the release of corticosteroids from the adrenal quarters as a result of colchicine addition appeared more remarkable when the tissue was incubated in the presence of ACTH (Fig. 5).

DISCUSSION

Colchicine administered s.c. to intact rats was found to be highly distributed to the anterior pituitary and adrenal glands (Table 1), suggesting the possibility that the agent is capable of altering adrenocortical function. In fact, corticosteroid levels in both the serum and the adrenal gland were remarkably increased by a subcutaneous injection of colchicine.
FIG. 2. Augmentation of adrenocortical function stimulated by ACTH administration in the rats pretreated with colchicine. One hr after a subcutaneous injection of colchicine (1 mg/kg), ACTH (0.1 U/rat) was injected s.c., followed by decapitation 30 min after the hormone injection (Group IV). Corticosteroid level in the serum was significantly higher than those in rats given ACTH alone (Group II) or colchicine alone (Group III). Corticosteroid level in the adrenal gland was also higher, though the augmentation was not statistically significant. Each column indicates the mean (N=5) and the vertical bar indicates the standard deviation.

FIG. 3. Comparison of efficacy between colchicine and ACTH in stimulation of adrenocortical function. Serum and adrenal corticosteroids 30 min after the injection of 0.1 U/rat of ACTH were much the same as these 2.5 hr after the subcutaneous administration of 1 mg/kg of colchicine (Group II vs. Group III). ACTH administration 2 hr after colchicine injection did not augment increases in corticosteroid levels elicited by the preinjection of colchicine (Group III vs. Group IV).
Fig. 4. Intracellular distribution patterns of corticosteroids in the adrenals of rats given colchicine, ACTH, and colchicine and ACTH. Rats in Group I-IV were treated in the same manner as in the previous experiments, the results of which are shown in Figs. 2 and 3. The adrenals of rats in each group were pooled, homogenized and preparatively centrifuged to obtain the subcellular fractions. Corticosteroids in the fractions were determined fluorometrically by the method previously described (10, 11).

(Figs. 1, 2 and 3). Although systemic administration of different chemical agents produces increases in serum and adrenal corticosteroids, their effect is temporary in almost instances, as, for example, in ACTH administration. In contrast to administration of these agents, colchicine administration produced long-lasting increases in serum and adrenal corticosteroids (Fig. 1). As colchicine has never been shown to affect the distribution, metabolism and excretion of corticosteroids in rats, its effect on corticosteroid levels is probably due to direct stimulatory action on the adrenal gland or to action on the anterior pituitary gland which stimulates ACTH secretion. The in vitro study of Temple and Wolff (8) indicated that antimicrotubular agents, including colchicine, acted directly on the cultured Y-1 adrenal tumor cells of mice and stimulated the synthesis and release of corticosteroids at very low concentrations such as \(8 \times 10^{-7} \, \text{M}\). In our in vitro study with the adrenal quarters of intact rats, low concentrations of colchicine did not affect the release of corticosteroids from adrenal gland, and only high concentrations of colchicine such as \(10^{-3} \, \text{M}\) clearly suppressed the release of corticosteroids from adrenal gland (Fig. 5). As pointed out by Temple and Wolff, the inhibitory effect of colchicine at high concentrations may be non-specific, therefore we think that colchicine given systemically may have no direct effect on adrenocortical
function in rats. The augmented increase in the level of corticosteroids to a maximum, as a result of colchicine administration in conjunction with ACTH administration (Fig. 2), and the similarity of the intracellular distribution pattern of corticosteroids in the adrenal gland between colchicine-treated rats and ACTH-treated rats (Fig. 4) indirectly indicate that colchicine administration stimulates ACTH secretion from the pituitary gland in rats. The stimulation of ACTH secretion results in promotion of adrenocortical function and elevates the levels of both serum and adrenal corticosteroids simultaneously. Long-lasting increases in serum and adrenal corticosteroids due to colchicine administration suggest the continuous stimulated secretion of ACTH after the administration of the drug. Kraicer and Milligan suggested that colchicine may possibly produce the augmented release of ACTH from the pituitary gland by continuously releasing a corticotropin-releasing factor from the hypothalamus in rats (4). Therefore, the continuous stimulation of synthesis and secretion of adrenocortical hormone due to colchicine may be one reasonable explanation for the effectiveness of colchicine in treating acute gouty arthritis.

**FIG. 5. In vitro effect of colchicine on corticosteroid release from the rat adrenal gland.** Ten pieces of the adrenal quarters of intact rats (30-35 mg) were incubated in a flask under the conditions described in Materials and Methods. Corticosteroids in the incubation media and in the subcellular fractions of the incubated tissue were determined. The first incubation (pre-incubation) was carried out for 30 min, followed by a second incubation with colchicine at concentrations of $10^{-5}$ to $10^{-3}$ M for 30 min. The third and final incubation was successively performed with or without ACTH (0.01 U/flask). Each point indicates the mean of the 3 incubations and the vertical bar indicates the standard deviation.
REFERENCES


