INHIBITION OF THE ACTION OF EXOGENOUS PARATHYROID HORMONE BY ITS AZO-DERIVATIVE IN PARATHYROIDECTOMIZED RATS

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The 100% azo-derivative of parathyroid hormone (PH) inhibits the action of exogenous PH in parathyroidectomized rats. This effect of the derivative is reflected much more in the blood calcium than in the blood phosphorus level.

The object of the investigation described below was to obtain a competitive anahormone of parathyroid hormone (PH), i.e., a derivative capable of preventing the action of the native hormone [2, 3].

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 80-100 g deprived of food and water for 24 h before the tests began. Parathyroidectomy was performed by thermocoagulation. Azotization of a lyophilized PH powder, with an activity of 20 units/mg, to the extent of 30 and 100% and 100% azotization of bovine serum albumin was carried out with novocainamide, diazotized with sodium nitrite, by the method previously developed in the laboratory [1]. The biological activity of the compounds was tested by injecting them subcutaneously immediately after the end of the operation; in some cases the tests were carried out 24 h after parathyroidectomy. The azo-compounds were dissolved in alkaline physiological saline and usually injected 1 h before injection of the native PH. Blood was taken by cardiac puncture 6 h after the beginning of the experiment. The serum calcium concentration was determined by photometric titration with Trilon B (EDTA) in the presence of murexide [4], and the serum phosphorus by the method of Karakashov and Vichev [5].

EXPERIMENTAL RESULTS AND DISCUSSION

The results given in Table 1 show that the 30 and 100% azo-derivatives of PH (A30-PH and A100-PH) were not themselves biologically active. However, A100-PH prevented restoration of the normal blood calcium level in the parathyroidectomized animals if this compound was injected 1 h before the active PH. The observed effect depended on the dose of azo-derivative injected. The competitive effect of the compound on the blood phosphorus level was much less marked than on the blood calcium.

Neither A30-PH nor the 100% azo-derivative of bovine serum albumin (A100-BSA) inhibited the action of native PH.

Similar results were obtained in animals undergoing parathyroidectomy 24 h before the beginning of the main experiment, with the slight exception that the effect of the operation and inhibition of the effect of the active hormone on the blood calcium level by the 100% azo-derivative of PH were slightly more marked than in the tests carried out immediately after the operation (6.46 ± 0.15 mg and 6.35 ± 0.05 mg% compared with 6.79 ± 0.07 and 6.77 ± 0.04 mg%; in the first case, $P > 0.05$; in the second case, $P < 0.01$).

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TABLE 1. Effect of Azo-Derivatives of PH on Blood Calcium and Phosphorus Concentrations (in mg%) in Parathyroidectomized Rats in Experiments Performed Immediately after the Operation (M ± m)

Procedure	No. of animals	Calcium	Phosphorus
Control (fasting)	12	9.46 ± 0.10	7.61 ± 0.26
Parathyroidectomy (PTE)	9	6.79 ± 0.07	9.70 ± 0.35
PTE + 20 units PH	10	9.00 ± 0.10	8.11 ± 0.37
PTE + 15 mg A30-PH	10	6.58 ± 0.08	9.18 ± 0.34
PTE + 15 mg A100-PH	10	6.65 ± 0.09	9.51 ± 0.36
PTE + 15 mg A30-PH + 20 units PH	15	9.21 ± 0.24	7.45 ± 0.23
PTE + 15 mg A100-PH + 20 units PH	14	6.77 ± 0.04	8.68 ± 0.29
PTE + 7.5 mg A100-PH + 20 units PH	5	8.64 ± 0.11	7.84 ± 0.53
PTE + 2.5 mg A100-PH + 20 units PH	5	9.12 ± 0.15	7.09 ± 0.39
PTE + 15 mg A100-BSA + 20 units PH	10	9.01 ± 0.11	7.69 ± 0.29

Despite the fact that both A30-PH and A100-PH were immunologically identical with the active hormone, only the 100% azo-derivative of PH (which, unlike A30-PH, inhibited the specific effect of the native hormone) can be regarded as its competitive anahormone. The absence of competitive properties in the 30% azo-derivative of PH can perhaps be explained by differences in the accumulation of the tested compounds in the target tissues (in particular, in the bones). It was noted that the ability of A100-PH to prevent the action of exogeneous PH is reflected to a greater degree in the calcium than in the phosphorus level, probably because of differences in determination of the properties in the native PH molecules [11, 12]. Since the results of the experiments carried out immediately after parathyroidectomy were virtually the same as those obtained 24 h after the operation, it can be assumed that the effect of thyrocalcitonin, the action of which is attributed by some writers [9, 10] to the character of the changes in the calcium and phosphorus levels observed immediately after thermocoagulation of the parathyroid glands, could hardly be reflected in the results now obtained.

Recent investigations have shown that PH, by its action on target tissues, activates their adenyl cyclase, leading to the accumulation of cyclic 3',5'-AMP (CAMP) [8]. It is now considered that the specific hormonal action is effected through this last compound [13]. Theophylline, which inhibits phosphodiesterase, an enzyme hydrolyzing CAMP [7], is known to restore the normal blood calcium level in parathyroidectomized rats [14]. In preliminary experiments undertaken to study the mechanism of action of A100-PH, the writers found that this derivative could not prevent the effect of theophylline. However, it cannot be concluded from these indirect experiments that A100-PH, or still more, other known anahormones, act on a later stage than CAMP. A solution to this problem may be obtained by the direct study of the effect of preliminary administration of A100-PH on the changes in adenyl cyclase activity produced by the action of native PH. Evidence that such an attempt would not prove fruitless or sufficiently specific is given by the fact that an ACTH analog without hormonal activity blocked the stimulation of adenyl cyclase activity induced by ACTH, but not that induced by noradrenalin or glucagon [6].

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