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Potentiation of medroxyprogesterone acetate antineoplastic activity by histidine in rat mammary tumours*

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Summary. The antitumour activity of arginine, histidine and medroxyprogesterone acetate (MPA) was studied in female rats with dimethylbenzanthracene (DMBA)-induced mammary adenocarcinomas. After 15 days of treatment, regression was observed in 4 of 19 (21%), 3 of 18 (16.7%) and 22 of 59 (37.3%) tumours taken from rats given arginine, histidine or MPA, respectively. A total of 17 rats with tumours that had been non-responsive to MPA were then treated with MPA plus histidine for 15 more days; the growth of 3 lesions (17.6%) was arrested, and 5 tumours (29.4%) regressed markedly. The antineoplastic activity of MPA was found to be related to the oestrogen- (ER) and progesterone-receptor (PgR) concentrations measured in the tumours before the start of treatment, whereas that of arginine and histidine appeared to be independent of receptor status. A significant reduction in serum prolactin (PRL) levels occurred in rats that were responsive to MPA alone or to MPA plus histidine. In tumours taken from the same rats, the PRL receptor content was also significantly increased in comparison with that in non-responsive tumours. In contrast, serum PRL levels increased significantly in rats with tumours that were non-responsive to MPA, whereas no change in serum PRL or PRL receptor levels was observed in rats treated with arginine or histidine. Histidine showed the ability to increase the number of ERs and PgRs in responsive tumours; this could have been responsible for the unexpected potentiation of MPA antineoplastic activity. In contrast, the levels of ER and PgR in uteri taken from the same rats were not modified. Furthermore, the addition in vitro of histidine to cytosols obtained from tumours of control animals did not influence ER and PgR concentrations. These results suggest that the effect of histidine on ER and PgR levels is probably specific for tumour tissue and is not due to a direct activity.

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Introduction

Medroxyprogesterone acetate (MPA) is a progestin that is widely used in the treatment of patients with advanced breast cancer. Its mechanism of action is still obscure but is related, at least in part, to its antioestrogenic activity, which is reflected in the rat by a reduction in the number of oestrogen receptors in target tissues and by changes in the structure and function of organs (pituitary gland, ovaries and adrenals) that are direct or indirect sources of oestrogens [4].

Regression of dimethylbenzanthracene (DMBA)-induced rat mammary tumours has been observed after MPA treatment in our previous investigations [5, 11]. On the other hand, a growth-inhibitory effect on this and other types of tumours by amino acids (mainly arginine) has been reported in past [1,7] and recent years [3, 15] in rodents, but its mechanism of action remains obscure.

In the present study we investigated the question as to whether some amino acids (namely, arginine and histidine) can inhibit the growth of rat mammary tumours induced by DMBA and/or increase the antiproliferative activity of MPA. In fact, we have previously shown that MPA increases cAMP levels in mammary tumours that are responsive to this progestin [12], and Cho-Chung et al. [3] have demonstrated that dibutyryl-cAMP inhibits the growth of mammary carcinomas in the rat and that this effect is enhanced by arginine.

Materials and methods

Drugs and radiochemicals MPA (Depo Provera) was purchased from Upjohn Italiana (Milan, Italy). Histidine, arginine and DMBA were obtained from Sigma (St. Louis, Mo. USA). [6,7-^{3}H]-Oestradiol-17β (40 Ci/mmol), 16alpha-ethyl-21-hydroxy-19-nor[6,7-^{3}H]pregn-4-en-3,20-dione (ORG 2058; 42 Ci/mmol) and unlabelled ORG 2058 were obtained from Amersham International plc (Bucks, UK) Unlabelled diethylstilboestrol (DES) was purchased from Prodotti Gianni (Milan, Italy).

Ovine prolactin (NIDDK oPRL-I-2, 35 IU/mg) was kindly provided by the Pituitary Hormone Distribution Program of the National Institute

of Diabetes and Digestive and Kidney Diseases (NIDDK; Bethesda, Md.). Ovine prolactin was iodinated (ovine prolactin labelled with iodine 125; sp. act., 40–57 mCi/mg) by the method of Bolton and Hunter [2], in which radioactive iodine is introduced by the reaction of free amino groups of the protein with *N*-succinimydil 3[4-hydroxy-5-(^{125}I)-iodophenyl] propionate (Amersham; Little Chalfont, UK). Rat prolactin labelled with iodine 125 (Sp. act., 47 mCi/mg) was purchased from New England Nuclear (Boston, Mass.).

Animals and treatments. Mammary tumours were induced in 50-day-old Sprague-Dawley female rats by a single oral dose of 20 mg DMBA in olive oil.

Experiments in vivo. Rats that developed mammary tumours ($n = 136$) were allocated at random to 4 groups and treated for 15 days as follows: first group (25 rats), 250 mg/kg arginine given i.p. daily; second group (25 rats), 250 mg/kg histidine given i.p. daily; third group (61 rats), 75 mg/kg MPA given i.m. daily. The remaining 25 rats acted as controls and were given saline (5 ml/kg daily, i.m.). The schedule of treatment and the dose of MPA were chosen according to previous studies that have shown a noticeable regression in >30% of mammary tumours after 15 days' administration of 75 mg/kg i.m., with no signs of general or local toxicity [5, 11]. The dose of arginine was that previously used by Cho-Chung et al. [3], and the dose of histidine was identical to that selected for arginine.

Tumour length and width were measured every 3–4 days with calipers, and treatments were initiated when the area measured approximately 1.5 cm^2. Growing and regressing tumours were differentiated from stable tumours by a positive or negative change, respectively, of >30% in the tumour area. Tumours were biopsied before the start of treatment using a UNICUT biopsyneedle (Angiomed, FRG) while rats were under light ether anesthesia. Tissue samples (about 100 mg) sufficient for determining both oestrogen and progesterone receptor levels (single-point assay) as well as for histopathological examination were taken.

The majority of the animals were killed by decapitation after 15 days of treatment (24 h after the last injection). A total of 17 rats that had shown no response to MPA were treated daily with MPA (75 mg/kg, i.m.) plus histidine (250 mg/kg, i.p.) for 15 additional days and then killed. In parallel, six rats with tumours that had not regressed after 15 days of MPA treatment continued to receive the same dose of MPA for 15 more days ("control" group with MPA alone). From some of the rats that had been killed, blood samples were collected for the determination of serum prolactin (PRL) concentration and for haematological and biochemical studies. Tumours were also removed and subjected to microscopic examination. In addition, PRL-receptor (PRL-R) levels were determined in the membranes. Physical appearance, behaviour and body weights were controlled daily in all animals.

In subsequent experiments in vivo, 50 rats with mammary tumours were treated with histidine (250 mg/kg daily i.p.) for 15 days. In seven tumours that were considered to be responsive to such treatment and in the other seven, which were non-responsive, oestrogen- (ER) and progesterone-receptor (PgR) levels were determined by Scatchard analysis. ERs and PgRs were also measured in uteri taken from the same tumour-bearing rats. After 24 h some of these animals were killed by decapitation and blood samples were collected for the measurement of serum oestradiol concentration.

Experiments in vitro. The effect of histidine on the binding of radioactive oestradiol and ORG-2058 to their specific cytosol receptors obtained from DMBA-induced mammary tumours (taken from control rats) was investigated in vitro to ascertain whether histidine has a direct activity on ERs and PgRs in these tissues.

Measurement of ERs and PgRs in tumours and uteri. Cytosolic ERs and PgRs were determined as previously described [4]. In brief, tissues were homogenized in TEG buffer [50 mM TRIS-HCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 12 mM thioglycerol, 250 mM sucrose and 10% glycerol; pH 7.4 at 4° C] using a Polytron PT-10 homogenizer. Homogenates were centrifuged at 1,000 *g* for 10 min at 4° C. The supernatant was then centrifuged at 105,000 *g* for 60 min at 4° C to obtain the cytosol. Duplicate samples of cytosol were incubated overnight at 4° C with varying concentrations (0.6–2.9 pmol) of radioactive oestradiol-17β or ORG 2058.

Non-specific binding was determined by parallel preincubation of a sample of cytosol with a 250-fold excess of unlabelled DES or ORG 2058 for 15 min at 4° C before the labelled hormones were added. After incubation, unbound hormone was removed by treatment with dextran-coated charcoal. The results, obtained by Scatchard analysis, were expressed as femtomoles of hormone specifically bound per milligram of cytosol protein. In the experiments performed on biopsies, a single-saturating-dose assay was used according to McGuire et al. [9]. The protein concentration in each sample of cytosol was determined according to the method of Lowry et al. [8].

In the in vitro experiments the interference of histidine with the specific binding of oestradiol or ORG 2058 to their receptors was determined by pre incubation of each sample of cytosol (150 µl) with 50 µl buffer containing histidine (instead of buffer alone) before the radioactive hormone was added. Values for non-specific binding were obtained by the addition of a 250-fold excess of unlabelled DES or ORG 2058 as described for in vivo experiments.

Measurement of membrane PRL-R levels

Membrane preparation. Tumours were homogenized in 0.3 M sucrose (10 vol.) at 4° C using a Polytron PT-10 homogenizer. The homogenates were centrifuged at 15,000 *g* for 20 min at 4° C and the resulting supernatant was decanted. This was centrifuged at 105,000 *g* for 60 min at 4° C to obtain a microsomal pellet, which was then resuspended in ice-cold 25 mM TRIS-HCl, 10 mM $MgCl_2$ (pH 7.4) using a hand-operated Potter-Elvehjem-type homogenizer. A sample of the membrane suspension was solubilized by incubation in 1 M NaOH for 60 min at 60° C for protein determination. The remainder was stored at –20° C at a protein concentration of 4 mg/ml for hormone-binding studies.

Hormone-binding studies. PRL-binding studies were performed according to Muccioli et al. [10]. In the binding assays, about 80,000 cpm [^{125}I]-PRL were added to each tube, containing 0.1 mg membrane protein in a final volume of 0.5 ml assay buffer (25 mM TRIS-HCl, 10 mM $MgCl_2$, 0.1% bovine serum albumin, pH 7.4). After 16 h of incubation at 20° C, bound and free radiolabelled hormone was separated by low-speed centrifugation (1,500 *g* for 30 min at 4° C). The supernatant was decanted and the radioactivity in the membrane pellet was counted in a Packard auto-gamma-counter. Specific binding was evaluated as the difference between binding in the absence of excess unlabelled hormone and binding in its presence (2 µg/ml), expressed as a percentage of the total counts added to incubation medium.

Measurement of serum PRL and oestradiol levels Serum PRL levels were determined by radioimmunoassay (RIA) as recommended by the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases; Baltimore, Md.). The validity of the assay has been tested by the measurement of PRL levels in the serum of ovariectomized or hypophysectomized rats. In these animals the values for serum PRL lie in the range of 5–7 and <2 µg/l, respectively. The intra- and inter-assay variabilities were 5% and 9.4%, respectively. Serum oestradiol levels were determined by RIA (Estradiol Double Antibody; Diagnostic Products Corporation, Los Angeles, Calif.) [17]. Ovariectomized rat serum was used as a test control, and spiked samples were used to validate the assay. With the same aim, the serum of rats in different stages of the oestrous cycle was used. The kit was equipped with standards that had oestradiol values ranging from 5 to 500 pg/ml. The intra-assay coefficient of variation at 23 pg/ml was 5%, and the inter-assay coefficient of variation at 94 mg/ml was 2.7%.

Haematological and biochemical studies. Leukocytes, erythrocytes, haemoglobin and haematocrit were evaluated using a Coulter counter, whereas differential leukocyte counts were obtained by the May-Grünwald-Giemsa method. Glucose, blood urea nitrogen, total proteins,

SGPT, SGOT, and cholesterol values were determined by spectrophotometric methods using Boehringer Mannheim tests (FRG).

Statistical analysis. All results were expressed as group arithmetic means ± standard deviation. Statistical comparisons between group means were carried out by analysis of variance (ANOVA). As regards the effects of arginine, histidine or MPA (alone or combined) on tumour growth, the differences between treatment groups were analyzed by both non-parametric and parametric ANOVA for repeated measures. Statistical analysis was performed according to a general linear-models procedure using an SAS system on an IBM 386 PC [13]. Given that the same significance was obtained with the different approachs, the mean and parametric results were used in the final version. Duncan's multiple-range test for variables was used to identify the significant time points in the study. For clarity, the results were also shown as the percentage of change over baseline values.

Results

The administration of DMBA induced the development of 121 adenocarcinomas and 15 fibroadenomas. The results reported herein concern only adenocarcinomas.

Tumour ER and PgR levels before treatment

As shown in Table 1, no appreciable difference existed in ER and PgR tumour content among the four groups of rats before the start of treatments.

Effect of arginine, histidine and MPA on tumour growth in vivo

Figure 1 shows the effects of arginine, histidine and MPA on tumour growth (19, 18 and 59 adenocarcinomas, respectively). After the 15th day of treatment, 4 of 19 (21%) and 3 of 18 (16.7%) tumours taken from rats given arginine and histidine, respectively, regressed (about 40% regression); 6 tumours (33.3%) from rats treated with histidine remained unchanged. After MPA treatment, 22 tumours (37.3%) regressed (about 75% regression), 8 (13.6%) remained static and 29 (49.1%) continued to grow. The majority of control tumours (24/25) grew steadily, and 1 remained static.

As reported in Table 2, the effect of arginine, histidine and MPA on tumour growth was statistically significant. On the other hand, tumours that responded to MPA therapy showed ER and PgR levels that were significantly higher

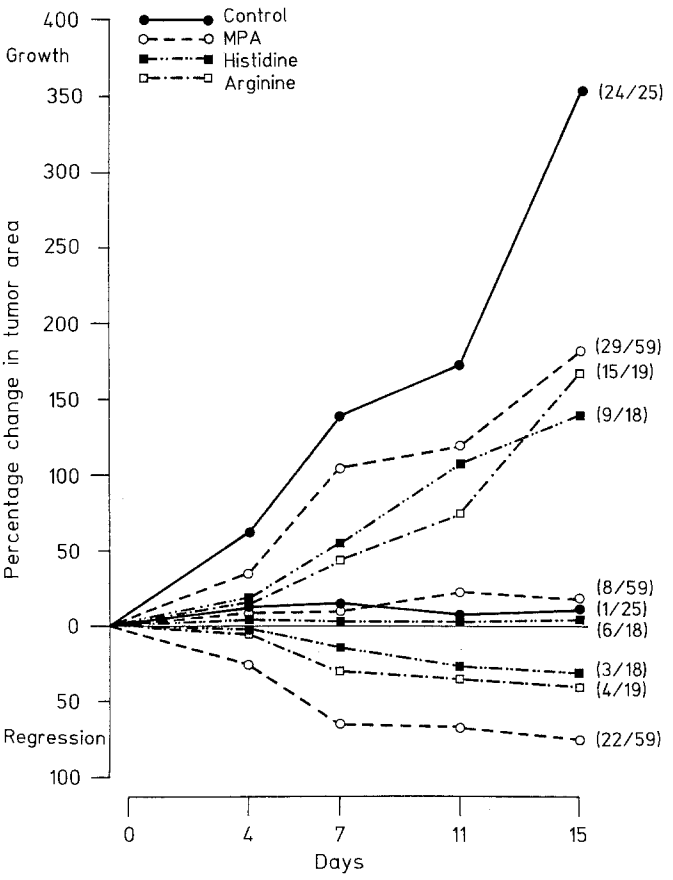

Fig. 1. Effects of MPA, histidine or arginine on growth of DMBA-induced mammary tumours in rats. Animals were killed by decapitation after 15 days of treatment (doses are reported in Materials and methods). *Numbers in parentheses* indicate the amount of tumours in each category

Table 1. Oestrogen and progesterone receptor levels in DMBA-induced mammary tumours determined before the start of treatments

Group	ER[a]	PgR[a]
Controls (25)	22.1±8.3	19.3±6
MPA (59)	22.4±10	17.2±7
Histidine (18)	20.0±10.2	20.1±7.3
Arginine (19)	24.1±12	19.6±5.5

[a] Expressed in fmol oestradiol or ORG-2058 bound/mg cytosol protein; single-point assay

Values represent means ± SD. Numbers in parentheses indicate the amount of cases in each group. ER, Oestrogen receptors; PgR, progesterone receptors

Table 2. Statistical analysis of tumour growth in rats treated with arginine, histidine or MPA for 15 days

Group	Mean tumour area at the following days of treatment (mm^2)				
	0	4	7	11	15
Controls (25)	158 ±11.8	253.3±63.6	372.8±129	417.8±139	688.1±226.1
MPA (59)	180.7±56.4	228.6±109.6*	267.3±210.2*	285.5±224.2*	334.3±299.9*
HISTIDINE (18)	169.7±43.8	192.3±62.4*	228 ±105.5*	283.2±161.8*	310.7±195.6*
ARGININE (19)	164.9±44	181.5±58.7*	204 ±104*	247 ±136.6*	343.6±230.2*

* Significantly different from controls values ($P < 0.05$ according to Duncan's multiple-range test). Effect of different treatments over time, $P = 0.0001$ (repeated-measures ANOVA); differences within responses to treatment over time, $P = 0.0001$ (repeated-measures ANOVA)

Table 3. Oestrogen and progesterone receptor levels in mammary tumours responsive or non-responsive to MPA, histidine or arginine

Tumour group	ER[a]	PgR[a]
MPA-responsive (22)	31.5±7.8*	23.7±4.7*
MPA-non-responsive (29)	14.7±5.5	11.6±3.9
Histidine-responsive (3)	21.6±9	11.7±3.5
Histidine-non-responsive (9)	19.8±10.2	17.8±4.4
Arginine-responsive (4)	19 ±4.1	13.2±3.6
Arginine-non-responsive (15)	25.4±13.1	20.6±5

[a] Expressed in fmol oestradiol or ORG 2058 bound/mg cytosol protein; single-point assay
* P <0.001 vs MPA non-responsive tumours
Values were measured before the start of treatments. Numbers in parentheses indicate the amount of cases in each group. ER, Oestrogen receptors; PgR, progesterone receptors

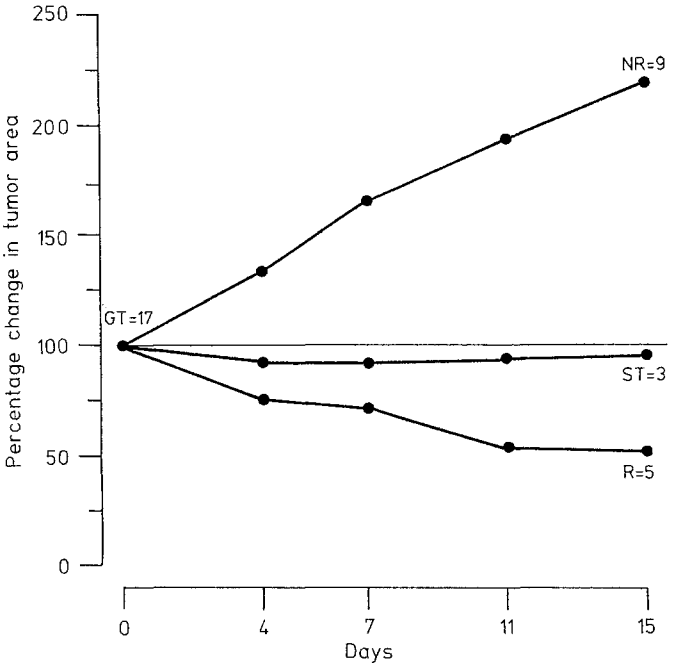

Fig. 2. Mean percentage of growth or regression of DMBA-induced mammary tumours from rats non-responsive to MPA alone (75 mg/kg i.m. for 15 days) that were subsequently treated with MPA (at the same dose) plus histidine (250 mg/kg i.p.) for the next 15 days. The area of tumours growing (*GT*) in spite of MPA therapy is taken as being equal to 100. *NR*, tumours nonresponsive to the new treatment; *R*, responsive tumours; *ST*, tumours that stopped growing and remained static after the new treatment. The areas of tumours taken from rats treated with MPA alone were quite similar to those of NR tumours; for purposes of clarity, these are not shown

than those in tumours that were non-responsive to this drug (Table 3). This result suggests that the antiproliferative effect of MPA is related, at least in part, to the ER and PgR levels measured in the tumours before treatment, in accordance with clinical findings. In contrast, tumours that regressed after histidine or arginine administration did not show such a correlation with ER and PgR status.

Only 6 of 29 rats with tumours that had been non-responsive to MPA were killed at this point; another 6 continued receive MPA alone for 15 more days (MPA-treated "controls"), whereas the remaining 17 were treated with MPA plus histidine for 15 additional days. No arrest or regression of tumour area was observed in the rats treated with MPA alone (Fig. 2). This result confirmed previous findings [11] that tumours failing to respond to MPA after 7–15 days of treatment are generally refractory to this drug even after more prolonged administration. In the 17 rats treated with MPA plus histidine, the growth of 3 tumours (17.6%) was arrested and 5 tumours (29.4%) regressed markedly (about 47% reduction in the tumour area; Fig. 2). The statistical analysis between the two

Table 4. Statistical analysis of tumour growth in rats non-responsive to MPA during the previous 15 days and then treated with MPA alone or MPA plus histidine for 15 additional days

Group	Mean tumour area (mm^2) at the following days of treatment				
	15	19	22	26	30
MPA (6)	503.3±144	683 ±157.6	806.5±147.1	1,072 ±188.4	1,064.5±182.6
MPA + Histidine (17)	508.8±274	552.2±383.5	633.9±457.1	719.3±511	776.5±564.4

Effect of different treatments over time, P = 0.0001 (repeated-measures ANOVA); differences within responses to treatment over time, P = 0.04 (repeated-measures ANOVA)

Table 5. Serum prolactin concentrations and specific binding of ovine prolactin labelled with iodine 125 to tumour membranes of female rats with DMBA-induced mammary tumours that were treated with MPA, histidine or arginine for 15 days

Group		Serum prolactin		Tumour prolactin-specific binding[a]	
		Non-responders (μg/l)	Responders (μg/l)	Non-responders	Responders
Controls		11.9±0.7 (13)		1.16±0.20 (10)	
MPA	75 mg/kg i.m.	19.9±2.7 (6)*	5.7±0.5 (13)**	1.02±0.24 (6)	1.90±0.12 (10)**
Histidine	250 mg/kg i.p.	11.1±0.6 (9)	12.2±0.8 (2)	0.96±0.16 (8)	1.12±0.16 (3)
Arginine	250 mg/kg i.p.	11.6±0.7 (15)	11.9±0.6)4)	1.10±0.16 (8)	1.16±0.12 (4)

[a] Expressed as a percentage of total radioactivity per 0.1 mg protein
* P <0.001 vs controls and responders; ** P <0.001 vs controls and non-responders
Values represent means ± SD. Numbers in parentheses indicate the quantity of animals in each group

Table 6. Serum prolactin concentrations and specific binding of prolactin to tumour membranes of rats with DMBA-induced mammary tumours non-responsive to MPA alone (75 mg/kg i.m. for 15 days) that were subsequently treated with MPA (at the same dose) plus histidine (250 mg/kp i.p.) for 15 more days

Group	Serum PRL (μg/l)	Tumour PRL-specific binding
Controls (6)	11.8±2.1	1.20±0.16
Non-responders (9)	16.7±3.5	1.20±0.18
Responders (5)	4.8±1.3*	2.45±0.41*

* P <0.001 vs controls and non-responders
Values represent means ± SD. Numbers in parentheses indicate the amount of cases in each groups. PRL, Prolactin

Table 8. In vitro effect of histidine on the binding of radioactive oestradiol or ORG 2058 (a synthetic progestin) to their specific cytosol receptors obtained from DMBA-induced rat mammary tumours

Drug concentration (nM)	ER[a]	PgR[a]
None	20 ±1.8	11.8±1.2
Histidine 1	20.6±1.6	12 ±0.8
Histidine 10	21.2±1.4	11.5±1
Histidine 100	18.4±2.2	12.2±1
Histidine 1,000	19.6±1.9	11.9±1

[a] Expressed in fmol hormone bound/mg cytosol protein
Values represent means ± SD of 6 determinations. Data were obtained by Scatchard analysis. Concentration of protein in the cytosol, 2.5–3.5 mg/ml Dissociation constants: ER, 0.12–1.8 nmol/l; PgR, 0.41–1.05 nmol/l. ER, oestrogen receptors; PgR, progesterone receptors

groups showed a significant difference between the group treated with MPA alone and that treated with MPA plus histidine (Table 4). In particular, a significant time-treatment interaction (P = 0.04) was found, and this suggests that the effect of histidine is synergistic [13].

As regards the toxicity of the treatments (both single and combined), no drug-induced changes were seen in the physical appearance, behaviour, body weights, or haematological and serum biochemical parameters of the animals (data not shown).

Effect of histidine, arginine, MPA and MPA plus histidine on serum PRL and PRL-R levels

A significant reduction in serum PRL levels occurred in rats that were responsive to MPA alone (Table 5) or to MPA plus histidine (Table 6). Moreover, the PRL-R content of tumours that were responsive to both of these treatments increased significantly in comparison with that of non-responsive tumours (Tables 5, 6). In contrast, neither tumour PRL-R contents nor serum PRL concentrations were modified in rats that were responsive to arginine or histidine alone (Table 5).

These results are in good agreement with those we obtained in a previous investigation in the rat, which indicate that low serum PRL concentrations and high PRL-R levels are markers of good response to endocrine therapy [5].

Effect of histidine on the levels of serum oestradiol, ER and PgR

The results reported in Table 7 demonstrate that after 15 days of treatment, histidine significantly increases the oestrogen- and progesterone-binding capacity of some DMBA-induced mammary tumours. In contrast, the dissociation-constant values were not substantially modified. The effect of histidine on ER and PgR levels did not seem to be influenced by oestradiol in the circulation, since the plasma concentrations of this hormone lay in the same range in responsive, non-responsive and control groups. Contrary to results obtained in tumour tissues, histidine did not induce changes in uterine ER and PgR concentrations (Table 7). As shown in Table 8, the addition in vitro of histidine to cytosols from mammary tumours (taken from untreated rats) did not modify the number of ERs and PgRs.

Table 7. Serum oestradiol concentrations and number of oestrogen and progesterone receptors in tumours and uteri of rats with DMBA-induced mammary tumours after 15 days of treatment with histidine (250 mg/kg daily i.p.)

Group	Serum oestradiol (pg/ml)	Tumour		Uterus	
		ER[a]	PgR[a]	ER	PgR
Controls (8)	25.2±15.4	20 ±13.3 (K_d, 0.40–1.30)[b]	17.2 ±8.5 (K_d, 0.70–1)	166.5 ±10 (K_d, 0.15–0.67)	178.4 ±8.5 (K_d, 0.6–1.5)
Non-responsive to histidine (7)	23.7±12.4	48 ±33.6 (K_d, 0.60–1.6)	35.1 ±17.8 (K_d, 0.13–1.8)	156.6 ±7 (K_d, 0.21–0.7)	170.2 ±9 (K_d, 0.8–1.2)
Responsive to histidine (7)	26 ±18.5	95.1 ±54.5* (K_d, 0.30–1.25)	84.5 ±54* (K_d, 0.25–0.96)	168 ±8 (K_d, 0.6 –1)	185 ±7.5 (K_d, 0.6–1)

[a] Expressed in fmol oestradiol or ORG 2058 bound/mg cytosol protein
[b] Expressed in nmol/l
* P <0.01 vs controls and P <0.05 vs non-responsive tumours
Values represent means ± SD. Numbers in parentheses indicate the amount of animals in each group. Receptor levels were obtained by Scatchard analysis. Concentration of protein in the cytosol, 2.5–3.5 mg/ml. ER, Oestrogen receptors; PgR, progesterone receptors

Discussion

The results of this study confirm the antineoplastic activity of both MPA and arginine in the rat and demonstrate for the first time that histidine has such activity. More notably, our experiments point out that histidine can markedly increase the antineoplastic effectiveness of MPA.

That MPA, like other endocrine treatments, is more frequently effective in ER- and PgR-rich human breast cancers has long been known [6]. Our present results show that this also holds true in DMBA-induced rat mammary tumours. They also suggest that tumours that fail to regress after MPA treatment (in spite of ER and PgR positivity) can achieve a good response to MPA through the induction of a significant enhancement of hormone-receptor levels, although the involvement of other mechanisms cannot be excluded.

On the other hand, the reason why ER and PgR levels are increased by histidine is not clear. This effect, which occurred only in tumours that were responsive to histidine, is related neither to prolactin (Table 5) nor to oestradiol in the circulation (Table 7). Furthermore, this increase in receptor levels does not seem to be due to a direct activity of histidine on ER and/or PgR, since the in vitro addition of histidine to cytosols from control tumours did not induce changes in the capacity of oestradiol and ORG 2058 to bind to their specific receptors (Table 8). Also obscure remain the mechanisms by which ER and PgR levels are increased by histidine in tumours but are not changed in uteri taken from the same rats (Table 7). A different response by mammary and uterine ERs to the same substance has previously been reported and might also be involved in this case. Indeed, Vignon and Rochefort [18] have found that prolactin increases the ER concentration in DMBA-induced mammary tumours but has no effect on that in the uteri of the same animals.

As regards tumour PRL-R levels, it is unlikely that their increase can result from lower circulating PRL levels, In fact, in previous experiments we have shown that in normal rats MPA induces a significant decrease in serum PRL levels, which is accompanied by a marked reduction (80%–90%) in PRL-specific binding in liver and ovarian membranes [4]. Moreover, in rats with DMBA-induced mammary tumours, the administration of MPA plus bromocriptine for 15 days significantly reduced PRL in the circulation of all animals treated, whereas the specific binding of PRL was low in non-responsive tumours and high in responsive lesions [5].

The increase in PRL-R concentrations that was seen only in tumours that were responsive to MPA alone (Table 5) or to MPA plus histidine (Table 6) may speculatively be interpreted as the result of a process of a reversible tumour-cell differentiation induced by the hormone. Indeed, several observations suggest that apart from an antimitotic effect, an increased maturation of cancer cells is involved in the mechanisms by which hormones induce cancer regression [14]. This enables the tumour cells to recover some features of normal mammary cells that are partially lost during the process of dedifferentiation, such as the ability to synthesize specific hormone receptors. According to Tisdale [16], this hormone-induced differentiation occurs only in the presence of a favourable situation, such as a suitable endocrine environment (in our study, the reduction of PRL in the circulation) or the presence of a high level of hormone receptors. The ability of histidine to increase the number of ERs and PgRs in mammary tumours could speculatively be related, at least in some cases (e.g. responsive tumours), to a process of cancer-cell maturation similar to that attributed to hormones.

In conclusion, our experiments point out that histidine can significantly increase the antiproliferative activity of MPA. It is very important that the histidine-induced increase in the antineoplastic activity of MPA in vivo occurred in a substantial percentage (about 30%) of tumours that were previously non-responsive to MPA alone. This finding also suggests that the lack of response of some breast tumours to endocrine treatments such as MPA could be related, at least in part, to a form of resistance (such as the masking of tumour hormone receptors) that may be overcome by histidine. It is certain that histidine can induce some rat tumours to respond to MPA that were previously non-responsive to this progestin.

Whether this effect of histidine also occurs in vivo in humans is not presently known. If this were the case, a remarkable advantage would undoubtedly be obtained for patients with breast cancer, since a potentiation and/or prolongation of the antineoplastic activity of MPA would be possible through the administration of a "drug" (i.e. histidine) that has very low toxicity and a low cost. This could also delay the use of aggressive antineoplastic therapies with cytotoxic drugs.

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References

1. Beard HH (1943) Effect of subcutaneous injection of individual amino acids upon the appearance, growth and disappearance of the Emge sarcoma in rats. Exp Med Surg 1: 123
2. Bolton AE, Hunter WM (1973) The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. Biochem J 133: 529
3. Cho-Chung YS, Clair T, Bodwin JS, Hill DM (1980) Arrest of mammary tumor growth in vivo by L-arginine: stimulation of NAD-dependent activation of adenylate cyclase. Biochem Biophys Res Commun 96: 1306
4. Di Carlo F, Racca S, Conti G, Gallo E, Muccioli G, Sapino A, Bussolati G (1984) Effects of long-term administration of high doses of medroxyprogesterone acetate on hormone receptors and target organs in the female rat. J Endocrinol 103: 287
5. Di Carlo F, Muccioli G, Bellussi G, Conti G, Racca S (1988) High tumour prolactin receptor content and lack of increase in serum prolactin levels as predictors of good response to endocrine therapy in rat mammary cancer. Int J Cancer 41: 767
6. Jensen EV (1981) Hormone dependency of breast cancer. Cancer 47: 2319
7. Levy HM, Montanez G, Feaver ER, Murphy EA, Dunn MS (1954) Effect of arginine on tumour growth in rats. Cancer Res 14: 198
8. Lowry OH, Rosebrough NJ, Farr AL, Randall KJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265

9. McGuire WL, De La Garza M, Chamness GC (1977) Evaluation of estrogen receptor assays in human breast cancer. Cancer Res 37: 637
10. Muccioli G, Ghe' C, Di Carlo R (1985) Drug-induced membrane modifications differentially affect prolactin and insulin binding in the mouse liver. Pharmacol Res Commun 17: 883
11. Racca S, Conti G, Crispino A, Gallo E, Di Carlo F (1985) Effects of medroxyprogesterone acetate on DMBA-induced mammary tumours. Chemioterapia 4: 236
12. Racca S, Conti G, Portaleone P, Di Carlo F (1988) Experimental studies on the mechanisms of the antineoplastic activity of medroxyprogesterone acetate. In: Biology and biochemistry of normal and cancer cell growth. Harwood Academic, London, p 173
13. SAS user's guide (1987) Statistic version 5th ed. SAS Institute, Inc., Cary, North Carolina
14. Stoll BA (1982) Perspectives on hormonal therapy in cancer. In: Furr BJA (ed) Clinics in oncology. W. B. Saunders, London, p 3
15. Takeda Y, Tominaga T, Tei N, Katamura M, Taga S, Murase J, Taguchi T, Miwatani T (1975) Inhibitiory effect of L-arginine on growth of rat mammary tumours induced by 7,12-dimethylbenz(a)anthracene. Cancer Res 35: 2390
16. Tisdale MJ (1982) Modulation of tumour growth. In: Stoll BA (ed) Endocrine relationships in breast cancer. Heinemann Medical, London, p 280
17. Ueno S, Kuroda T, MacLaughlin DT, Ragin RC, Manganaro TF, Donahoe PK (1989) Mullerian inhibiting substance in the adult rat ovary during various stages of the estrous cycle. Endocrinology 125: 1060
18. Vignon F, Rochefort H (1976) Regulation of estrogen receptors in ovarian-dependent rat mammary tumours: I. Effects of castration and prolactin. Endocrinology 98: 722