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FENOTEROL EFFECTS ON THE *IN VITRO* IMMUNE RESPONSE

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Abstract — Beta-2-adrenergic agonists are often employed in the treatment of acute bronchostenosis. Following our recent investigations into the influence of some drugs (cromolyn, ketotifen, theophylline) on the immune response, in this study we analyzed the *in vitro* effects of fenoterol (β-2-adrenergic agonist) on the immune response.

The mitogen-(PHA)-induced proliferation of peripheral mononuclear cells (PMNC), the PMNC proliferation induced by anti-T3 and anti-T11 monoclonal antibodies (MAbs), the PHA-induced lymphokine — interleukin 2 (IL-2) and interferon-gamma (IFN-γ) — production were studied in ten healthy volunteers. Since the plasmatic peak of fenoterol following a single inhalation of 200 μg is about 20 ng/ml, in the experiments herein reported the drug was tested in the cultures at concentrations lower, equal and higher than the plasmatic peak: respectively, 2, 20 and 200 ng/ml.

Furthermore, for a more detailed study of T-lymphocyte activities, we also evaluated the effect of fenoterol on T-cell clone proliferation.

Our results, which reveal no effects of fenoterol on the studied immunological parameters, acquire relevance when related to our previous reports showing a depression of the immunological response exerted by theophylline and ketotifen.

The β2-symphathomimetics are important and commonly employed drugs in the treatment of bronchostenotic episodes. Among these compounds, fenoterol hydrobromide (chemically it resembles orciprenaline) is reported as selectively acting on β2-adrenoreceptors.

We recently demonstrated that some drugs commonly used in the treatment of broncho-obstructive syndromes — such as cromolyn (Canonica, Ciprandi, Bagnasco & Scordamaglia, 1986), ketotifen (Ciprandi, Scordamaglia, Ruffoni, Pizzorno & Canonica, 1986) and theophylline (Scordamaglia, Ciprandi, De Palma, Simonassi, Cinquegrana & Canonica, 1986; Scordamaglia, Ciprandi, Ruffoni, Caria, Paolieri, Venuti & Canonica, 1987) — may influence the immune response. Following these investigations, in the present study we analyzed the effects of fenoterol on: mitogenic-induced (phytohemagglutinin — PHA) proliferation of peripheral mononuclear cells (PMNC), anti-T3 monoclonal antibody (MAb)-induced PMNC proliferation, PHA-induced lymphokine (interleukin 2, IL-2, and gamma-interferon, IFN-γ) production and PHA-induced proliferation of T-cell clones.

In addition, since different T-cell activation pathways through various membrane structures, such as the T11 complex, were recently described (Meuer, Hussey, Fabbi, Fox, Acuto, Fitzgerald, Hodgon, Protentis, Schlossma & Reinherz, 1984), we also tested the fenoterol effects in this *in vitro* assay, in order to obtain further information concerning a possible effect of the drug on T-cell activation and functions.

EXPERIMENTAL PROCEDURES

Patients

Ten healthy young volunteers aged from 21 to 25 years (mean 22 years), six males and four females, were studied.

Fenoterol

Since the pharmacological plasmatic peak level of fenoterol is about 20 ng/ml after 200 μg inhaled (Laros, VanUrk & Raminger, 1977), we employed pure fenoterol (kindly provided by Boehringer Ingelheim, Florence, Italy) in the cellular cultures at the following concentrations: 2, 20 and 200 ng/ml.

PMNC isolation

PMNC were isolated by a Ficoll – Urovison gradient from 20 ml of heparinized blood from each subject and then washed three times in culture medium (RPMI 1640 + 10% heat-inactivated foetal calf serum from Flow Laboratories + 2 mmol glutamine + 40 μg/ml gentamicin).

PHA-, anti-T3 and anti-T11 MAbs-induced PMNC proliferation

PMNC were set up in triplicate for 48 h (72 h for cultures with anti-T11 MAb) at concentrations of 200,000 PMNC/well in 0.2 ml, using round-bottomed microtiter plates (Sterilin), in culture medium (CM) both with and without mitogens (PHA: 1% *v/v*; MAb T3: 50 ng/well; MAb T11: 1/60,000 plus phorbol – myristate – acetate (PMA) = 10^{-9}M). Plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Moreover, PMNC proliferations were evaluated both with and without fenoterol. Each well was pulsed with ^{3}H-thymidine (TdR) (1 μCi/well) 18 h before being harvested on glass wool filters using an automated cell harvester (Titertek D-001, Flow Labs, Scotland) and then ^{3}H-TdR incorporation was measured in a beta-counter (Beckman, Fullerton CA).

PHA-induced cultures for IL-2 and IFN-γ production

PMNC (2 million in 2 ml) were cultured in tubes with CM supplemented with PHA both with and without fenoterol (20 ng/ml). After 24 h, supernatant fluids were collected and centrifuged for lymphokine assay.

IL-2 assay

IL-2 activity in PMNC culture supernatants was assessed using the CTL-L (IL-2 dependent murine line) by the microassay described by Gillis, Ferm, Ou & Smith (1978). Briefly, 100 μl of sample culture supernatants were added to 5000 CTL-L/well. After 24 h [^{3}H]-TdR was added. After a further 6 h the cultures were harvested and counted in a beta-counter as described above. Five thousand CTL-L/well plus 100 μl of CM were used as a negative proliferative control. Data were expressed as percentages of the maximal CTL-L proliferation provided by mitogen-induced IL-2-producing Jurkat-FHCRL cell line supernatant.

IFN-γ assay

Gamma-interferon concentration in PMNC culture supernatants was measured by a specific solid-phase immunoradiometric assay (IMRX Centocor Inc., U.S.A., kindly provided by Medical System Spa, Genoa, Italy).

An anti-γ-IFN monoclonal antibody was used both coating glass beads and as a radiolabeled "second antibody". Results were calculated as IFN-γ units (U) by means of the curve obtained by serial dilutions (4 – 50 U/ml) of a standard IFN preparation (one unit refers to the NIH reference unit for gamma-interferon). All samples were assayed in the same run and the intraassay coefficient of variation was less than 5% at a concentration of 25 U/ml.

Isolation and cloning of T-lymphocytes

PMNC were isolated from peripheral blood of three different healthy volunteers by density gradient as described above, then rosetted with neuraminidase-treated sheep erythrocytes and purified by centrifugation over standard Ficoll – Urovison gradients. E-rosetting cells were >95% pure. Purified T-cells were diluted in CM and seeded at 0.25, 0.5, 1 and 2 cells/well in round-bottomed microtiter plates containing 50,000 irradiated (5000 rad) spleen feeder cells/well in CM plus PHA, 1% *v/v* Supernatant (SN) derived from cultures of PHA-stimulated human spleen (routinely deprived of PHA) was used as a source of IL-2. Such SN was added after 48 h of culture at a 50% (*v/v*) concentration. Plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Microcultures were supplemented on day 7 with 10^5 irradiated feeder cells resuspended in 100 μl of IL-2 SN and on day 14 with 100 μl of IL-2 only. Control wells contained irradiated feeder cells cultured in the presence of PHA and IL-2 SN, without addition of responder cells. After 14 – 18 days of culture each microwell was assessed microscopically for growth.

The proliferating microcultures characterized to be T4-positive and IL-2 producers were then divided into several microwells for the following assay concerning the effect of fenoterol on PHA- and anti-T3 MAbs induced T-cell clone proliferation.

Assay of PHA- and anti-T3 MAbs-induced proliferation of T-cell clones

T-lymphocytes were cultured as described for PMNC.

Statistical analyses

The Student's *t*-test was used.

Table 1. PHA-, anti-T3 and anti-T11 MAbs-induced PMNC proliferation at three different concentrations of fenoterol in cultures

Fenoterol	PHA	anti-T3	anti-T11
0 ng/ml	75,135 ± 9665	14,342 ± 1187	61,824 ± 10,039
200 ng/ml	74,718 ± 15,706	12,888 ± 2702	60,251 ± 11,149
20 ng/ml	79,112 ± 12,630	14,088 ± 2229	61,499 ± 9930
2 ng/ml	81,496 ± 13,907	11,712 ± 1546	66,208 ± 7667

Data express the mean ± S.E.M. of counts/min in ten cases.
Fenoterol 0 ng/ml vs 200, 20 and 2 ng/ml, respectively, both for PHA, anti-T3 and anti-T11 MAbs. P = N.S. (Student's t-test).

Table 2. PHA-induced lymphokines productions with and without fenoterol

	Without	With
Interleukin-2	21.31 ± 4.1	22.17 ± 3.3
Interferon-γ	35.71 ± 6.2	31.89 ± 5.7

Data express the mean ± S.E.M. of CTL-L proliferation percentage for IL-2 and of Units for IFN-γ, both in ten cases.
Without fenoterol vs fenoterol 20 ng/ml : P = N.S. (Student's t-test).

Table 3. Effect of fenoterol on PHA-induced proliferation of T-cell clones

Fenoterol	PHA
0 ng/ml	16,118 ± 4380
200 ng/ml	13,821 ± 2261
20 ng/ml	14,112 ± 3744

Results express the mean ± S.E.M. of counts/min in six clones. Fenoterol 0 ng/ml vs 200 and 20 ng/ml:
P = N.S. (Student's t-test).

RESULTS

As reported in Table 1, both PHA-, anti-T3 and anti-T11 MAbs induced *in vitro* PMNC proliferations were unaffected by adding fenoterol to cultures at any concentration (2, 20 or 200 ng/ml).

Lymphokine production induced by PHA also appeared to be unaffected by fenoterol (Table 2).

Finally, PHA-induced proliferation of T-cell clones did not significantly differ by adding fenoterol (Table 3).

DISCUSSION

Bronchostenosis is a clinically relevant symptom frequently associated both with immunophlogosis (such as allergic reactions) and infectious states, and is often characterized by a depression of the immune system.

We previously demonstrated an inhibitory effect of ketotifen (Ciprandi *et al.*, 1986) and theophylline (Scordamaglia *et al.*, 1986; Scordamaglia *et al.*, 1987) on some parameters of the immune response. While ketotifen showed a significant inhibitory effect on autologous mixed lymphocyte reaction responsiveness, theophylline appeared to significantly reduce the PMNC proliferation induced by both PHA and anti-T3 MAbs at culture concentrations equal to (15 μg/ml), lower (5 μg/ml) and higher (30 μg/ml) than the therapeutic blood level of the drug. Therefore, in the light of these previous studies, we decided to evaluate the influence of fenoterol on some *in vitro* immunological parameters.

As far as T-cell proliferation is concerned, we studied different activation pathways. In fact, we used both anti-T3 MAbs, acting through the complex T3/Ti (i.e. T-cell receptor) and PHA, recently shown to be effective partially through the same membrane structure (Moretta *et al.*, 1986).

Any influence on T-lymphocyte proliferation has been observed using all these pathways.

Since the data already referred to were obtained by using bulk culture, we decided to approach the problem in a more selective manner. With this in mind we studied the possible influence of fenoterol on T-cell clones, which were derived from any single cell of the peripheral blood, according to the method described by Moretta *et al.* (1983). These experiments demonstrated that fenoterol does not affect T-cell clone proliferation.

In addition, PHA-induced lymphokine production (i.e. IL-2 and IFN-γ) is unaffected by fenoterol addition.

The experimental data provided in the present report might, *per se*, be considered uninteresting, but they gain remarkable importance when evaluated in the context of our previous reports concerning the

inhibitory effects of theophylline and ketotifin on the immune response.

In fact, since there is almost always involvement and frequently impairment of the immune system during broncho-obstructive states, the employment of a drug, which has no effect on the immune response, is clinically suggested.

In addition, since cromolyn exerts an enhancing effect on the *in vitro* immune response (Canonica *et al.*, 1986), from a clinical viewpoint it may be fruitful to associate inhaled cromolyn with fenoterol solution in the same aerosol preparation in order to achieve a dual effect both on the bronchial motor tone and on the immune response.

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