SIGNIFICANCE OF ATP-SPLITTING ACTIVITY OF RAT PERITONEAL MAST CELLS IN THE HISTAMINE RELEASE INDUCED BY EXOGENOUS ATP

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It has previously been reported that exogenous adenosine-5'-triphosphate (ATP) evokes a marked histamine release from isolated rat mast cells with accompanying morphological changes including degranulation when Ca²⁺ is present in the medium (1, 2). Since it has been suggested that the energy requiring process is involved in the histamine release (3, 4), it seems to be reasonable to assume that ATP can be utilized as a source of energy supply in the process of histamine release induced by this compound, and that Ca²⁺ plays a role as a cofactor of ATPase of mast cell membrane. Diamant and Krüger (5, 6) and Diamant (7) who observed ATP-induced histamine release stated a possibility that Ca-activated "ecto-ATPase" in the mast cell membrane plays an important role in this histamine release.

The present investigation was undertaken to see whether inorganic phosphate can be liberated from ATP when histamine release was induced by ATP from the mast cells in the presence of Ca²⁺ and whether mast cells are responsible for the 5'-nucleotidase activity in this respect, in order to know if the exogenous ATP really serves for the energy source in the histamine release.

MATERIALS AND METHODS

Preparation of cell fractions. The peritoneal fluid of Wistar rats (250-300 g, males), obtained as described before (2), was placed gently over the two layers of gum arabic solutions of different specific gravities, 1.060 and 1.070, which were dissolved in 0.9% NaCl in a conical centrifugation tube. Centrifugation was made at 650 x g for 10 minutes. Mast cells were precipitated beneath the lower layer of gum arabic. They were collected with a pipette and used as the mast cell fraction. The other cells were concentrated in the interphase between the upper and lower layers. They were mononuclear cells, lymphocytes, granulocytes and a few mast cells, of which more than 80% were mononuclear cells and lymphocytes. Hereafter the suspension of these mixed cells is referred to as the macrophage fraction. The mast cells and macrophages (cells of macrophage fraction) obtained from 10-15 rats were washed 3 times with 6 ml of the incubation medium described below and resuspended in the same medium and used for each experiment. The mast cells in samples were counted in a hemocytometer after staining with toluidine blue. The protein contents of the cell fraction were determined by the method of Lowry et al. (8).
Experiments on 5'-nucleotidase activity. Mast cells or macrophages (0.4-0.6 mg protein per tube) were suspended in 1.9 ml of the incubation medium containing 154 mm NaCl, 0.5 mm CaCl₂ or MgCl₂, 0.05 % bovine serum albumin (BSA), and 10 mm Tris-HCl (pH 7.3). For the experiments using medium of pH 6.0 or 8.5, 10 mm Tris-maleate was used as the buffer. The cell suspension was preincubated for 5 minutes at 37°C and then added with 1 mm ATP or other 5'-nucleotides; the final volume was adjusted to 2.0 ml. After incubation for 10 minutes, the reaction was terminated by the addition of ice-cold trichloroacetic acid (8%, final). After centrifugation at 3,000 × g for 15 minutes, inorganic phosphate (Pi) in the supernatant was determined by the method of Martin and Doty (9) modified by Ernster and Lindberg (10). In order to examine the effect of cell disruption on the nucleotidase activity of the mast cells and macrophages, freezing (dry ice-methanol) and thawing (37°C) of the cell suspension was repeated 3 times before the incubation with ATP. The effect of compound 48/80 was studied by preincubating the cells with this compound (1 µg/ml) for 5 minutes at 37°C prior to ATP addition. The effect of previous treatment at 45°C for 5 minutes, which inhibits the histamine release by ATP, was also examined for the ATPase activity of the cell suspensions.

ADP-ATP exchange reaction. The ADP-ATP exchange reaction was investigated in the mast cell fraction by the procedure described by Wadkins and Lehninger (11). Each sample of reaction mixture contained 2 mm ATP, 2 mm ADP (0.5 μC), 154 mm NaCl, 1 mm CaCl₂, 10 mm Tris-HCl (pH 7.3), 0.05 % BSA and mast cells (0.4-0.6 mg protein ÷ 2–3 × 10⁸ mast cells); the final volume was 0.5 ml. The reaction was initiated by adding the ¹⁴C-ADP-ATP mixture at 37°C, and after 5-minute incubation it was terminated by the addition of 0.1 ml of 2.5 N perchloric acid. After centrifugation (3,000 × g, 15 minutes), the nucleotides in the supernatant were separated by paper chromatography and each nucleotide spot cut out, and eluted with deionized water. The radioactivity of the eluate was measured by a gas flow counter.

Experiments on histamine release. Histamine release was examined at 37°C for 10 minutes on the same sample of mast cell fraction which was used for the determination of nucleotidase activity. Before the addition of trichloroacetic acid, 0.1 ml of the reaction mixture containing about 2 × 10⁷ mast cells was poured into 1.9 ml of incubation medium previously chilled in ice water, and centrifuged at 3,000 × g for 15 minutes. Histamine in the supernatant and sediment was assayed on atropinized guinea-pig ileum. All the values of histamine were expressed in terms of the base.

Experiments on Ca-ion binding. Mast cells (0.5 mg protein) were incubated in 2 ml of the medium containing 154 mm NaCl, 1 mm ATP, 0.5 mm CaCl₂ with 0.02 μC/ml of ⁴⁰Ca, 0.05 % BSA and 10 mm Tris-HCl (pH 7.3). In some experiments the mast cells were disrupted previously by freezing-thawing or by treating with compound 48/80 (1 µg/ml) at 37°C for 5 minutes. After the incubation of mast cells at 0°C or 37°C for 10 minutes, the reaction mixture was centrifuged at 3,000 × g for 15 minutes. Supernatant and precipitate were dissolved in 1 ml of 30 % H₂O₂ solution containing 0.1 ml of 62 % HNO₃, in a boiling bath. The radioactivity of the supernatant and precipitate was measured by
a gas flow counter.

**Chemicals.** Nucleotides and bovine serum albumin (Fraction V) were obtained from Sigma Corporation and Armour Pharmaceutical Company, respectively. Compound 48/80 was kindly supplied by Burroughs Wellcome & Co., Tukahoe, New York. \(^{14}C\)-ADP and \(^{45}CaCl_2\) were purchased from the Radiochemical Centre, Amersham, England.

**RESULTS**

**Liberation of Pi from ATP by mast cells and macrophages.** ATP was incubated in the presence of mast cell and macrophage fractions. As shown in Table 1, Pi liberation from ATP was enhanced by the addition of Ca\(^{2+}\) as well as Mg\(^{2+}\) in either fraction. Histamine release by ATP from mast cells was increased by the presence of Ca\(^{2+}\) but not increased by Mg\(^{2+}\) as described before (2). The stimulated Pi liberation elicited in the presence of Ca\(^{2+}\) or Mg\(^{2+}\) was much less marked in the mast cell fraction than in the macrophage fraction. It deserves to note that MgCl\(_2\) (5 mM) clearly inhibited the ATP-induced histamine release from mast cells in the CaCl\(_2\) (0.5 mM) containing medium, while Mg\(^{2+}\) did not inhibit but slightly increased Pi liberation in the mast cell as well as in the macrophage fraction in the same medium. Ba\(^{2+}\) stimulated the histamine release by ATP from mast cells, but in either cell fraction Ba\(^{2+}\) did not stimulate Pi liberation. EDTA (0.5 mM), which inhibited the ATP-induced histamine release from mast cells (2), also inhibited Pi liberation in both cell fractions.

**TABLE 1. Effect of Ca\(^{2+}\), Mg\(^{2+}\), Ba\(^{2+}\) and EDTA on Pi liberation from ATP (1 mM) in the mast cell and the macrophage fractions, and on histamine release from the mast cell fraction.**

| Addition (mm) | Pi liberated (pmoles/min/mg protein) | Mast cell fraction | Macrophage fraction | Histamine release (%) |
|--------------|-------------------------------------|--------------------|---------------------|----------------------|
| None         | 13                                  | 43                 | 8                   |
| CaCl\(_2\)   | 0.5                                 | 25                 | 300                 | 77                   |
| MgCl\(_2\)   | 0.5                                 | 29                 | 318                 | 5                    |
| CaCl\(_2\)   | 0.5                                 | 32                 | 358                 | 35                   |
| MgCl\(_2\)   | 5.0                                 | 8                  | 38                  | 42                   |
| BaCl\(_2\)   | 0.5                                 | 2                  | 8                   | 3                    |

The values are shown as mean of 3 experiments. Each sample of the mast cell fraction was contaminated with macrophages, 10-15% of total cell counts.

**Nucleotide specificity.** As reported in the previous paper (2), in the presence of Ca\(^{2+}\), ATP induced histamine release from mast cells with concomitant degranulation. However, these reactions were specific only for ATP and 2'-deoxyadenosine-5'-triphosphate (dATP), and other nucleotides such as inosine-5'-triphosphate (ITP), guanosine-5'-triphosphate (GTP), adenosine-5'-diphosphate (ADP) and adenosine-5'-monophosphate (AMP) were all ineffective in both reactions, histamine release and degranulation. In this connection, the nucleotidase activity in both mast cell and macrophage fractions was
studied for the substrate specificity. As shown in Table 2, Pi was liberated also from ITP, GTP, ADP and AMP when incubated with Ca\(^{2+}\) in both fractions. All these nucleotidase activities were again much higher in the macrophage fraction than in the mast cell fraction.

**Table 2. Nucleotidase activity of the mast cell and the macrophage fractions in the presence of CaCl\(_2\) (0.5 mm).**

| Nucleotide (1 mm) | Pi liberated (µmoles/min/g protein) |
|-------------------|-------------------------------------|
|                   | Mast cell fraction | Macrophage fraction |
| ATP               | 25 | 300 |
| ITP               | 11 | 230 |
| GTP               | 8  | 187 |
| ADP               | 21 | 208 |
| AMP               | 18 | 185 |

The values are mean of 3 experiments. Each sample of the mast cell fraction was contaminated with macrophages, 10-15% of total cell counts.

**Table 3. Liberation of Pi from ATP (1 mm) in the mast cell and the macrophage fractions in the presence of CaCl\(_2\) (0.5 mm), and histamine release from the mast cell fraction, under various conditions.** Either fraction was incubated at 37°C for 10 minutes.

| Pretreatment or Condition | Pi liberated (µmoles/min/g protein) | Histamine release (%) |
|---------------------------|-------------------------------------|-----------------------|
|                           | Mast cell fraction | Macrophage fraction | Mast cell fraction |
| 37°C                      | 22 | 308 | 77 |
| 45°C*                     | 23 | 325 | 6  |
| Freeze-thawing            | 18 | 189 | 95 |
| Comp. 48/80               | 21 | 298 | 75 |
| pH 6.0                    | 17 | 268 | 8  |
| pH 8.5                    | 21 | 315 | 11 |

* Pretreated at 45°C for 5 minutes, then incubated at 37°C for 10 minutes.

The values are mean of 3 experiments. Each sample of the mast cell fraction was contaminated with macrophages, 10-15% of total cell counts.

**Liberation of Pi under various conditions.** The liberation of Pi from ATP in both cell fractions was examined in the presence of Ca\(^{2+}\) under various conditions. The results are summarized in Table 3. When mast cell and macrophage fractions were preincubated at 45°C for 5 minutes, the liberation of Pi was not much different from those preincubated at 37°C, whereas histamine release from the mast cell fraction was clearly inhibited by the pretreatment at 45°C for 1 minute (2). After both fractions were submitted to freezing and thawing, the ATPase activity of these cells was only slightly diminished, while this treatment exhausted practically all the histamine stores of mast cells. After the treatment with compound 48/80 (1 µg/ml) the enzyme activity was virtually unchanged in both fractions, whereas histamine release amounted to 75% in the mast cell fraction. The histamine release by ATP from mast cells was largely inhibited at both pH 6.0 and 8.5 (2), but the liberation of Pi was not much decreased in both fractions at these two pH's.
Contamination of macrophages in mast cell fraction. Fig. 1 shows the relationship between the Pi liberation and the protein content in both cell fractions. In this data both fractions were obtained from the same pooled sample. The Pi liberation is hardly detectable in the mast cell fraction if the protein equivalent is less than 150 µg. Even if the fraction contains a large amount of mast cells such as 370 µg protein, which corresponds to $2 \times 10^6$ cells, the ATP-hydrolysing activity is less than one tenth of that found in the macrophage fraction of the same protein amount. A linear relationship existed between the liberation of Pi and the amount of macrophages up to 370 µg of protein and this linear curve was highly reproducible with different samples. But the liberation of Pi from ATP in mast cell fraction varied considerably with different samples. Now a question arises as to whether the contamination of macrophages is responsible or not for the activity of Pi liberation in the mast cell fraction. Table 4 shows that the liberation of Pi in the mast cell fraction does not increase along with the increase in the protein content of this fraction but is clearly in parallel with the contamination of macrophages in this fraction. This may support the reality of the point in question.

**Fig. 1. Liberation of Pi from ATP in the mast cell and the macrophage fractions.** Both cell fractions were incubated at 37°C for 10 minutes with ATP (1 mM) in the medium containing CaCl$_2$ (0.5 mM). The mast cell fraction was contaminated with macrophages, about 10% of total cell count.

**Table 4. Contamination of macrophages in the mast cell fraction and liberation of Pi from ATP (1 mM) in the presence of CaCl$_2$ (0.5 mM).**

| Exp. No. | Protein (µg) | Macrophages in mast cell fraction (%) | (µmole Pi/min/g protein) |
|----------|--------------|-------------------------------------|--------------------------|
| 1*       | 1.52         | 5                                   | 8                        |
| 2        | 0.72         | 8                                   | 15                       |
| 3        | 0.68         | 14                                  | 21                       |
| 4        | 0.51         | 13                                  | 17                       |
| 5        | 0.50         | 33                                  | 42                       |
| 6        | 0.37         | 25                                  | 45                       |
| 7        | 0.31         | 11                                  | 17                       |

* Isolation procedure was repeated once more.
ADP-ATP exchange reaction: Experimental results mentioned above seem to indicate that the possibility of Pi liberation from exogenous ATP by the intact mast cells is extremely small. However, as alternative explanation for the difficulty in detecting released Pi, it may be possible that liberated Pi might be utilized for ATP resynthesis at the cell membrane. Attempting to study this possibility the catalysis of ADP-ATP exchange by the mast cells was examined. 

\[ ^4C-ADP-ATP \text{ mixture was added to the incubation medium containing } \text{Ca}^{2+}, \text{ with or without mast cells. Percentage of incorporation of radioactivity in ATP was } 4.0 \pm 0.35 \text{ (mean with S.E. from 5 experiments) in the presence of mast cell fraction whereas it was } 2.7 \pm 0.36 \text{ in the absence of mast cell, indicating practically no increase in this catalysis by mast cells.} \]

\[ \text{Ca binding}. \text{ In order to study the role of Ca ions in the ATP induced histamine release, } \text{CaCl}_2\text{-containing CaCl}_2 \text{ was added to the incubation medium containing mast cells, with or without ATP. As shown in Table 5 the binding of } ^{45}\text{Ca} \text{ by mast cells was not increased in the presence of ATP at } 0 \text{ C but it increased at } 37 \text{ C when ATP was present. However, a similar increase in } ^{45}\text{Ca} \text{ binding by mast cells was also observed when the mast cells were previously disrupted by the treatment with compound } 48/80 \text{ or with freezing-thawing even in the absence of ATP. In addition, when } ^{45}\text{Ca} \text{ was added to the mast cells previously degranulated by ATP in the presence of unlabelled } \text{Ca}^{2+}, \text{ the mast cells still bound } ^{45}\text{Ca} \text{ to a similar amount. These findings allow the explanation that } ^{45}\text{Ca} \text{ can be taken nonspecifically by the disrupted mast cells involving } ^{45}\text{Ca} \text{ exchange for unlabelled } \text{Ca}^{2+}. \]

**DISCUSSION**

The ATPase activity of the mast cell is reported to be demonstrable histochemically in rat peritoneal mast cells (12, 13) and in mast cells of various animals (14, 15). Schauer and Fider (16) stated that such an ATPase activity is found in the areas corresponding to the localization of metachromatic granules. Recently, Diamant and co-workers (7, 17,
reported that the mast cell membrane contains an "ecto-ATPase" which is stimulated by either Ca\(^{2+}\) or Mg\(^{2+}\), and that this enzyme activity is possibly involved in both processes of histamine release from and sodium uptake by the mast cells induced by exogenous ATP.

Despite these reports, the results of the present study revealed unexpectedly a low ATPase activity in intact mast cells. By the density gradient method using the two layers of gum arabic solution with different specific gravity, rat peritoneal cells were separated into the mast cell fraction and the macrophage fraction (mononuclear cells, lymphocytes, granulocytes, etc.). The Pi-liberation from added ATP in the incubation of these fractions was extremely marked in the macrophage fraction while it was surprisingly feeble in the mast cell fraction. The enzyme activity of the mast cell fraction did not parallel the protein content in the fraction but it increased in increasing amount of macrophages mixed in the mast cell fraction. Therefore, it is very likely that ATPase activity in the mast cell fraction is owed largely to the activity of macrophages contaminated in the mast cell fraction. Since even when the isolation procedure of mast cells was repeated, the mast cell fraction was still contaminated with macrophages in 5\% of total cell count, it is quite difficult to isolate peritoneal mast cells without any contamination of other cells as far as the density gradient method is used. In this connection, it becomes difficult to agree that the ATPase activity of isolated mast cells so far reported was due to the enzyme activity proper to the mast cells. There is no evidence for the existence of membrane-bound ATPase in intact mast cells. On the other hand, North (19) showed that ATPase activated by Ca\(^{2+}\) or Mg\(^{2+}\) was localized in the plasma membrane of guinea-pig peritoneal macrophages and polymorphonuclear leucocytes, and he suggested that ATPase at the macrophage membrane might play a function in phagocytosis.

The results of the present experiments suggest that ATPase activity of mast cells isolated so completely as to contain no macrophages may be so minute that it would be impossible to detect the activity by the available assay methods. The enzymatic properties of ATPase found in the mast cell fraction were entirely identical with those of ATPase of the macrophage fraction. Thus, ATPase detected in either of these two fractions showed the following properties: 1) it liberates Pi from various nucleoside 5'-triphosphate other than ATP and also from ADP and AMP, 2) the ATPase activity is activated by either Ca\(^{2+}\) or Mg\(^{2+}\), 3) the ATPase activity is not altered even after treatment of freezing-thawing or with compound 48/80, and does not undergo any change even when treated at 45\,C for 5 minutes or at pH 6.0 or 8.5, while Ca-ATP induced histamine release is inhibited by these treatments. Since ADP-ATP exchange reaction was not catalyzed by the mast cell fraction, the Pi liberation can be taken as the criterion of ATPase activity. On the basis of these properties it may be considered that the ATPase detected in the present experiment is identical with ATPase of mast cells reported by Diamant (7).

Now, supposing this nucleotidase to be "ecto-ATPase" localized in the plasma membrane, it is quite unreasonable to consider that this enzyme is involved in the Ca-dependent histamine release mechanism induced by extracellular ATP. One of the reasons for this contention lies in its enzymatic properties. First of all, ATP-induced histamine release...
is stimulated by Ca\(^{2+}\), and the effect of Ca\(^{2+}\) (0.1 mM) is competed by Mg\(^{2+}\) at high concentration (more than 0.5 mM), but the stimulating effect of Ca\(^{2+}\) (0.5 mM) in the ATPase activity is further augmented by Mg\(^{2+}\) at high concentration (5 mM). Ba\(^{2+}\) (0.5 mM) stimulates the ATP-induced histamine release whereas it does not stimulate the ATPase activity. In addition, the ATPase activity is not affected to any appreciable degree by the temperature or the pH at which ATP-induced histamine release is inhibited. Now, there will arise the following argument concerning the irrelevancy between ATPase activity and histamine release. Namely, these inhibitory factors or conditions might be associated with the step later than the action of ATPase in the plasma membrane in the processes of histamine release. However, the problem that is still more difficult to explain is as follows. As already mentioned in a previous report (2), since the histamine release induced by Ca-ATP is inhibited by various inhibitors of metabolic enzyme, it is thought to be dependent on the endogenous energy production by the mast cells. This is also true with many other histamine releasing agents, e.g. compound 48/80, toluidine blue, sinomenine, and antigens or antiserum (3, 4, 20–23). This indicates that even if it is possible for extracellular ATP to supply energy to the mast cell by the action of "ecto-ATPase", it would have no direct association with energy dependent histamine release mechanism or energy requiring process.

Uvnäs et al. (24, 25) proposed that the histamine release induced by compound 48/80 is a two-step process: an energy-requiring degranulation and a histamine release from the granules. The latter process is a non-enzymatic ion-exchange between granular histamine and extracellular cations. This proposal is accepted to be analogous to other chemical mediators by some investigators (26, 27). However, recently evidence has been presented that a degranulation is not obligatory in the release of histamine (20, 28, 29) or of serotonin from the mast cells (30). Such a finding seems to further weaken the contention that the histamine release mechanism is an energy requiring process. Hence it would seem possible to accept the concept proposed by Yamasaki (31) that "ability" of energy production is necessary for the mast cell to maintain its vital conditions, e.g. the structures or arrangement of molecular subunits in the membrane, required for the capacity in responding to the triggering action of histamine releasers. In this sense, the term "energy dependent" would be more suitable than "energy requiring" for the histamine release process.

**SUMMARY**

Rat peritoneal cells were divided into two parts of mast cell and macrophage fractions. ATP-splitting activity was very feeble in the mast cell fraction in contrast to the extraordinarily high activity in the macrophage fraction. In either fraction Pi-liberation from ATP was stimulated by Ca\(^{2+}\) as well as by Mg\(^{2+}\). This effect of Ca\(^{2+}\) was affected neither by the pH's nor by the temperature at which the ATP-induced histamine release is inhibited. ATP-splitting activities of both fractions were not altered after the treatment with compound 48/80 or after freezing-thawing. In the mast cell fraction this
activity did not parallel the protein content but increased in increasing number of other mixed cells (macrophages), that is, the contamination of macrophages is largely responsible for the ATP-splitting activity in the mast cell fraction. Therefore, ATP-splitting activity does not correlate to the ATP-induced histamine release.

"C-ADP-ATP exchange was not catalyzed by intact mast cells. "Ca bound to mast cells in the presence of ATP but a similar binding could be observed in mast cells previously exposed to compound 48/80 or to freezing-thawing.

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REFERENCES

1) Sugiyama, K. and Yamasaki, H.: Jap. J. Pharmac. 19, 175 (1969)
2) Sugiyama, K.: Jap. J. Pharmac. 21, 209 (1971)
3) Diamant, B. and Uvnäs, B.: Acta physiol. scand. 53, 315 (1961)
4) Säeki, K.: J. J. Pharmac. 14, 375 (1964)
5) Diamant, B. and Krüger, P.G.: Acta physiol. scand. 71, 291 (1967)
6) Diamant, B. and Krüger, P.G.: J. Histochem. Cytochem. 16, 707 (1968)
7) Diamant, B.: Int. Archs Allergy appl. Immun. 36, 3 (1969)
8) Lowry, O.H., Rosenhrough, J., Farr, A.L. and Randall, R.J.: J. biol. Chem. 193, 265 (1951)
9) Martin, J.B. and Doty, D.M.: Anal. Chem. 21, 965 (1949)
10) Ernster, L. and Lindberg, O.: Methods of biochemical analysis, vol. III, p. 16 (1956)
11) Wadkins, C.L. and Lehninger, A.L.: J. biol. Chem. 233, 1589 (1958)
12) Keller, R.: Nature, Lond. 196, 281 (1962)
13) Schafer, A.: Die Mastzelle, Gustav Fisher Verlag, Stuttgart (1964)
14) Eder, M. and Schafer, A.: Beitr. path. Anat. 124, 251 (1961)
15) Schauff, A. and Eder, M.: Klin.Wschr. 39, 76 (1961)
16) Schauke, A. and Eder, M.: Virchows Arch. path. Anat. Physiol. 335, 72 (1962)
17) Diamant, B.: Acta pharmac. tox. 25, suppl. 4, 33 (1967)
18) Dahlquist, R. and Diamant, B.: Acta physiol. scand. suppl. 330, 61 (1969)
19) North, R.J.: J. Ultrastruct. Res. 16, 83 (1966)
20) Yamasaki, H. and Komoto, S.: Int. Archs Allergy appl. Immun. (in press, 1971)
21) Yamasaki, H. and Säeki, K.: Proc. Japan Acad. 41, 958 (1965)
22) Moty, I. and Ishii, T.: Br. J. Pharmac. Chemother. 15, 82 (1960)
23) Keller, R. and Schward-Speck, M.: Int. Archs Allergy appl. Immun. 19, 202 (1961)
24) Uvnäs, B. and Thon, I.L.: Proc. Int. Wenner-Gren Center Symposium Series, vol. 5, p. 361 (1965)
25) Thon, I.L. and Uvnäs, B.: Acta physiol. scand. 71, 303 (1967)
26) Douglas, W.W.: Br. J. Pharmac. Chemother. 34, 451 (1968)
27) Rubin, R.P.: Pharmac. Rev. 22, 389 (1970)
28) Smith, C.: Am. J. Physiol. 193, 573 (1958)
29) Bloom, G.D. and Chakrabarty, N.: Acta physiol. scand. 78, 410 (1970)
30) Carlsson, S.A. and Ritzen, M.: Acta physiol. scand. 77, 449 (1969)
31) Yamasaki, H.: Proc. 17th Jap. Med. Congr. 1, 555 (1967)