catalytic hydrogenation of *o*-nitrobenzylideneaniline give any 2-phenylindazole.

The poor results from the reduction of the *o*-nitrobenzylamines *1* and the ready formation of the indazoles *2* from the *o*-nitrobenzylideneamines *4* may indicate an imine to be an important intermediate in the reductive cyclisation of *o*-nitrobenzonitrile to indazole.

The described reactions offer new syntheses of indazole and 2-substituted indazoles from readily available starting materials.[4] It may also be possible to use such reactions for the formation of further substituted indazoles.

Experimental. The general instrumentation has been described.[2] NMR signals are given in δ values. The products described were identified by comparison with authentic samples or by their spectroscopic properties.

Syntheses of 2-substituted indazoles (2). The imine *4* was dissolved in diethyl ether (200 ml) and dripped into a refluxing suspension of LAH (2 g) in diethyl ether (200 ml) during 40 min. After 1 h continued reflux, the product was obtained by hydrolysis of the reduction mixture followed by chromatography (dry column, silica gel, chloroform). 2-Phenylindazole (*2a*) (74 % yield) had m.p. 82 – 82.5 °C (lit.[5] 83 – 84 °C) and gave a picrate with m.p. 91 °C (lit.[6,7] 93 – 94 °C). 2-Methylindazole (*2b*) (71 % yield) had m.p. 53 °C (lit.[8] 56 °C). UV (EtOH):[9] λ_{max} 268 nm, ε_{max} 590 m^2 mol^{-1}; λ_{max} 288, ε_{max} 550. IR: 3100, 3050, 2950, 1630, 1520, 1390, 1300, 1160;, 1010, 910, 820, 790, 760 cm^{-1}. NMR ($CDCl_3$): δ 3.84 (3 H, s), 6.4 – 7.8 (5 H, m). 2-Octylindazole (*2c*) (70 % yield). UV (EtOH): λ_{max} 268 nm, ε_{max} 700 m^2 mol^{-1}; λ_{max} 286, ε_{max} 660. IR: 3050, 2950, 1660, 1620, 1500, 1460, 1150, 750 cm^{-1}. NMR ($CDCl_3$): δ 0.85 (3 H, t), 1.0 – 1.3 (10 H), 1.9 (2 H m), 4.2 (2 H, t), 6.8 – 7.8 (5 H, m), MS: *m/e* 230 (58 %, $C_{15}H_{22}N$).

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1. Behr, L. C. In Wiley, R. H., Ed., *Pyrazoles, Pyrazolines, Pyrazolidines, Indazoles and Condensed Rings*, Interscience, New York 1967.
2. Bakke, J. M. *Acta Chem. Scand. B 28* (1974) 645.
3. Rylander, P. N. *Catalytic Hydrogenation over Platinum Metals*, Academic, New York 1967.
4. Dayagi, S. and Degani, Y. In Patai, S., Ed., *The Chemistry of the Carbon-nitrogen Double Bond*, Interscience, New York 1970.
5. Paal, C. *Ber. Deut. Chem. Ges. 27* (1894) 2897.
6. Freundler, P. *Bull. Soc. Chim. Fr.* (3) *29* (1903) 742.
7. Lazaris, A. Y. *Zh. Org. Khim. 2* (1966) 1322.
8. v. Auwers, K. and Düesburg, M. *Ber. Deut. Chem. Ges. B 53* (1920) 1179.
9. Rosseau, V. and Lindwall, H. G. *J. Amer. Chem. Soc. 72* (1959) 3047.

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Adenylyl Cyclase in Isolated Plasma Membranes of Granulation Tissue

MARJUT VIHERSAARI, PIRJO LEHTINEN and EINO KULONEN

Department of Medical Chemistry, University of Turku, SF-20520 Turku 52, Finland

In an earlier paper [1] we have reported on the activities of 5′-nucleotidase (EC 3.1.3.5), Na^+,-K^+-activated Mg^{2+}-dependent adenosine triphosphatase (EC 3.6.1.3) and leucine-β-naphthylamidase (EC 3.4.11.1) in the plasma membranes isolated from experimental granulation tissue and then incubated in various conditions. The purpose of this note is to extend the observations to adenylyl cyclase (EC 4.6.1.1).

The purification of the adenylyl cyclase activity in the plasma membranes was of the same magnitude (18 ×) as calculated from the activities of 5′-nucleotidase, Na^+,K^+-activated Mg^{2+}-dependent adenosine triphosphatase and leucine-β-naphthylamidase [2] and slightly higher in the preparations from mature (3 week) granuloma than from proliferating (1 week) tissue (Table 1). Adenylyl cyclase is relatively insoluble;[3] only about 10 % of the total activity could be solubilized from the membranes with Lubrol WX.[4] This is in agreement with the earlier observations.

Cyclic AMP (10^{-3} – 10^{-6} M) stimulated by 15 – 30 % the activity of 5′-nucleotidase [5] of the plasma membrane both in incubated slices and isolated membrane preparations. Neuraminidase (EC 3.2.1.18) (12.5 U/sample) inhibited the activity of adenylyl cyclase in plasma membranes by 20 – 30 %, whereas hyaluronidase (EC 4.2.99.1) and collagenase (EC 3.4.4.19) were without any effect. Serotonin stimulated the

Table 1. Activity of adenylyl cyclase in various preparations of homogenized granulation tissue. Activities are expressed in (μmol cAMP formed)/(mg protein/min) and the figures are means of duplicates.

Subcellular fraction	Age of granuloma 7 d	21 d
Whole homogenate	0.6	–
7000 *g* supernatant	0.4	4.6
7000 *g* sediment	1.8	–
< 20 % sucrose	0.7	1.1
20 – 28 % sucrose [a]	3.8	8.1
28 – 38 % sucrose [b]	11.0	13.3

[a] "Light" plasma membranes. [b] "Heavy" plasma membranes (the bulk).

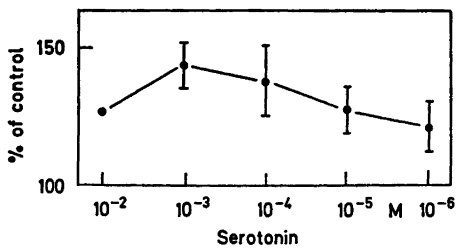

Fig. 1. Effect of added serotonin on the activity of adenylyl cyclase in incubated plasma membranes. For the details see the text. The means ± S.E.M. ($n = 5-8$, except $n = 2$ at 10^{-2} M) are given.

activity of adenylyl cyclase in incubated isolated plasma membranes (Fig. 1). The combined action of PGE_1 (15 μg/sample) and serotonin (10^{-3} M) was about 2-fold the straight summation. All the other additions mentioned in Table 1 of the earlier paper [1] were found to have no effect on the activity of adenylyl cyclase.

It is relevant that cyclic AMP is known to increase the synthetic functions in connective tissue [6-8] and that serotonin has been claimed to stimulate connective tissue both to proliferate [9] and to synthetize collagen.[10]

Experimental. The production of experimental granulation tissue by the subcutaneous implantation of viscose-cellulose sponges has been described previously [11] as have the details of the preparation of plasma membranes.[1,2]

For the assay of adenylyl cyclase, 150 μl of plasma membrane suspension were added to a substrate solution containing 1 mM unlabelled ATP, 45 pM ATP-^{14}C (25 μCi or about 3×10^4 cpm), 10 mM NaF, 10 mM theophylline, 3.3 mM $MgSO_4.7H_2O$ dissolved in 40 mM tris-HCl buffer, pH 7.3, to make a final volume of 600 μl. The temperature was 30 °C and the reaction time 10 min. When the effects of the additions were being studied, the added substances were preincubated with the plasma membrane preparation for 60 min. The enzyme action was stopped by immersing the tube into a boiling-water bath for 3 min and then cooling it in ice-cold water. The precipitated protein was removed by centrifugation (10 000 rpm, 10 min) and the supernatant analyzed for cAMP according to Krishna *et al.*[12] The sample was fractionated on Dowex 1-x8 (200/400 mesh, Cl^--form)-columns (0.6 × 3.5 cm). The ATP and other impurities in the sample were first eluted with 20 ml of water and then cAMP with 0.2 N formic acid. For the assay of radioactivity 200 μl of the sample were mixed with 10 ml of hydrophilic scintillation fluid [13] and counted with a Packard Model 3320 Liquid Scintillation Spectrometer. Protein was determined according to the method given by Lowry *et al.*[14] and its concentration in the sample was usually adjusted to 333 μg/ml.

Special reagents were obtained from various sources: cAMP, Fluka (Buchs, Switzerland); ATP, Fluka, or E. Merck (Darmstadt, Germany); AMP E. Merck; theophylline, Sigma (St. Louis, Mo. USA); serotonin Fluka; prostaglandin PGE_1, Upjohn; neuraminidase from *Vibrio cholerae*, 500 U/ml, Behringwerke (Marburg/Lahn, Germany), Code ORKD; adenosine-^{14}C (U)-5′-triphosphate CFB. 91 and adenosine/base-^{14}C(U)-3′,5′-cyclic phosphate, CFA. 442 from The Radiochemical Centre, Amersham, Bucks., U.K.

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1. Lehtinen, P., Känkänen, L. and Kulonen, E. *Acta Chem. Scand. B 29* (1975) 963.
2. Lehtinen, P., Vuorio, E. and Kulonen, E. *Biochem. J. 146* (1975) 565.
3. Sutherland, E. W., Rall, T. W. and Menon, T. *J. Biol. Chem. 237* (1960) 1220.
4. Vihersaari, T. *Unpublished work.*
5. Michell, R. H. and Hawthorne, J. N. *Biochim. Biophys. Acta 249* (1965) 333.
6. Ehrlich, H. P. and Bornstein, P. *Nature New Biology 238* (1972) 257.
7. Harper, E. and Toole, B. P. *J. Biol. Chem. 248* (1973) 2625.
8. Castor, C. W., Smith, S. F., Ritchie, J. C. and Scott, M. E. In Kulonen, E. and Pikkarainen, J., Eds., *Biology of Fibroblast*, Academic, London and New York 1973, p. 483.
9. Boucek, R. J. and Alvarez, T. R. *Science 167* (1970) 898.
10. Aalto, M. and Kulonen, E. *Biochem. Pharmacol. 21* (1972) 2835.
11. Viljanto, J. and Kulonen, E. *Acta Path. Microbiol. Scand. 56* (1962) 120.
12. Krishna, G., Weiss, B. and Brodie, B. B. *J. Pharmacol. Exp. Ther. 163* (1968) 379.
13. Juva, K. and Prockop, D. J. *Anal. Biochem. 15* (1966) 77.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem. 193* (1951) 265.

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