REFERENCES

1. C. F. Wilkinson, K. Hetnatski, G. P. Cantwell and F. J. DiCarlo, *Biochem. Pharmac.* **23**, 2377 (1974).
2. *Br. Pat.* 1364312 (1971), *Chem. Abstr.* **82**, 4255c (1975).
3. S. D. Atkin, B. Morgan, K. H. Baggaley and J. Green, *Biochem. J.* **130**, 153 (1972).
4. C. Hansch and T. Fujita, *J. Am. Chem. Soc.* **86**, 5175 (1964).
5. A. Leo, C. Hansch and D. Elkins, *Chem. Rev.* **71**, 525 (1971).

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Aggregation of blood platelets by adrenaline and its uptake

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In a previous communication the strong inhibitory action of dihydroergotamine (DHE) and BOL 148 on the adrenaline-induced aggregation of rabbit blood platelets was described[1]. For DHE the data revealed a competitive and for BOL 148 a non-competitive type of inhibition. The mechanism of the adrenaline-induced aggregation of blood platelets has not yet been clarified. In the case of serotonin-induced aggregation a relationship between the uptake of this biogenic amine by blood platelets and the aggregation seems to exist[2–4]. A similar connection is believed to exist for the adrenaline-induced aggregation[5, 6], however, it was not confirmed by other authors[7, 8].

In the present investigation we studied concomitantly the time course of adrenaline-induced aggregation of rabbit blood platelets and the uptake of adrenaline by platelets. Furthermore, the influence of DHE and BOL 148 on these reactions has been examined.

For reasons of comparison with the previous study we have chosen the same test design, and the isolation of rabbit blood platelets as well as registration of aggregation were performed as described in the preceding paper[1]. The blood platelets were resuspended in a modified Tris-buffered Tyrode's solution[9]. The aggregation of blood platelets was induced after incubation with 10^{-6} M serotonin (5-hydroxytryptamine creatinine sulfate, Fluka AG, Buchs SG, Switzerland) by [^{14}C]adrenaline [DL-adrenaline [*carbinol*-^{14}C]DL-bitartrate, The Radiochemical Centre, Amersham, England, spec. act. 50 mCi/mmole] at a final concentration of 5×10^{-6} M at 22–24°. Following the addition of labelled adrenaline, the change in optical density of the platelet suspension was recorded. One, 3, 5, 7, and 10 min later the samples were cooled in an ice bath and centrifuged.

To estimate the [^{14}C]adrenaline uptake the platelet pellets each containing $1{\cdot}76 \times 10^9$ blood platelets were washed one time with Tyrode's solution and then lysed with 1 ml distilled water. To 0·5 ml of each lysate 9·5 ml of a scintillation solution of the following composition was added: 7·0 g PPO, 50 mg POPOP, 50 g naphthalene, 20 ml methanol, dioxane ad 1000 ml. The radiochemical activity was measured by means of an LKB-Wallac 81 000 Automatic Liquid Scintillation Counter. After estimation of the quenching parameters of the solutions, the results were obtained as dis/min.

To control samples [^{14}C]adrenaline was added at 4° and the suspensions were centrifuged immediately. Under these conditions no uptake of adrenaline by blood platelets occurs[10]. Therefore, the radioactivity found in these samples was subtracted from that of the experimen-

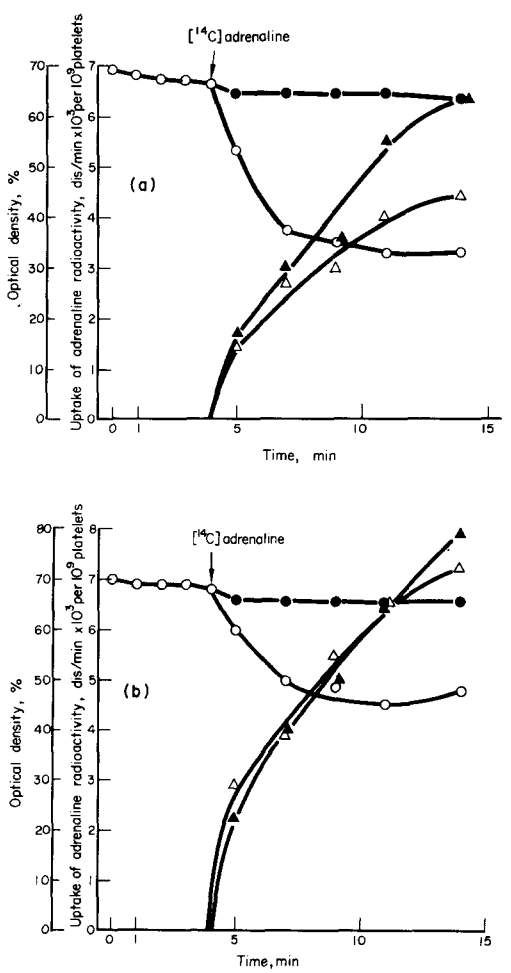

Fig. 1. Aggregation of rabbit blood platelets in Tris-buffered Tyrode's solution (pH 7·4, 22–24°) by 5×10^{-6} M [^{14}C]adrenaline and its uptake under the influence of (a) 10^{-11} M DHE, (b) 3×10^{-6} M BOL 148. Aggregation and [^{14}C]adrenaline uptake, resp., of the blood platelets in the absence (○, △) and in the presence of inhibitor (●, ▲). Each value represents the mean from five to eight experiments. For the sake of clarity standard deviations of the means have been omitted.

tal samples. To inhibit the aggregation of blood platelets at the beginning of the experiments DHE (Dihytamin®, VEB Arzneimittelwerk Dresden, German Democratic Republic) or BOL 148 (bromolysergic acid diethyl amide, Forschungsabteilung Sandoz AG, Basel, Switzerland) at concentrations of 10^{-11} M and 3×10^{-6} M, resp., were added.

Addition of [^{14}C]adrenaline results in aggregation of blood platelets which reaches a maximum value within 3–5 min. The uptake of adrenaline by the blood platelets starts immediately after the addition and, although the platelets are aggregated, it proceeds at the same velocity (Figs. 1a and b). In additional experiments adrenaline uptake was measured over a longer incubation time (180 min) under similar conditions but without stirring the suspensions. A linear increase in adrenaline radioactivity in the blood platelets was found. In 10 rabbits the uptake amounted up to 267 ± 197 dis/min per 10^9 blood platelets per min (mean ± standard deviation). This value corresponds to $2{\cdot}42 \pm 1{\cdot}79$ pmoles [^{14}C]adrenaline /10^9 blood platelets per min. The considerable spreading of the values reflects remarkable individual differences between the uptake capacities for adrenaline of the blood platelets in the several animals.

While adrenaline-induced aggregation of blood platelets is completely inhibited under the influence of DHE and BOL 148 (Figs. 1a and b), there are no significant differences in [^{14}C]adrenaline uptake of the experimental samples compared with that of non-treated controls. The apparent increase in adrenaline uptake after 10 min in the presence of DHE does not show statistical significance.

The present data do not favour the assumption that the adrenaline-induced aggregation of rabbit blood platelets is caused by the uptake of this amine. The binding of [^{14}C]adrenaline to the platelets is unchanged, while the aggregation is completely blocked by both drugs. From the adrenaline radioactivity found in the blood platelets at the time of maximum aggregation it can be concluded that no more than 15000 to 30000 molecules of adrenaline are bound to one blood platelet.

The competitive nature of the inhibition of adrenaline-induced aggregation by DHE[1] allows the conclusion that adrenaline and DHE compete for a common, at this time only hypothetical, receptor at the platelet surface which is responsible for the aggregation of blood platelets by adrenaline. Since during incubation with 10^{-11} M DHE only 15 molecules of inhibitor are available for each platelet, the possibility of inhibiting the aggregation by a few molecules of DHE reflects the extraordinarily strong affinity between this drug and the adrenaline receptor and allows the conclusion that the amount of adrenaline necessary for the induction of aggregation is extremely small.

The linear increase in adrenaline radioactivity in blood platelets observed after prolonged incubation is in accordance with the findings of Born and Smith[10] that adrenaline uptake of human blood platelets, at least at adrenaline concentrations up to 10^{-4} M in the incubation medium, does not show saturation kinetics. In this way, adrenaline uptake differs substantially from serotonin uptake. BOL 148 inhibits adrenaline-induced aggregation of blood platelets only at higher concentrations and non-competitively[1]. Contrary to DHE, it seems to influence adrenaline-induced aggregation by a rather unspecific mechanism. From data obtained with human blood platelets it is apparent that BOL 148 acts by influencing the second phase of adrenaline-induced aggregation representing the release reaction of endogenous ADP* which triggers the further aggregation of blood platelets[11, 12].

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REFERENCES

1. W. Barthel and F. Markwardt, *Biochem. Pharmac.* **23**, 37 (1974).
2. H. R. Baumgartner and G. V. R. Born, *J. Physiol., Lond.* **201**, 397 (1969).
3. H. R. Baumgartner, *Thromb. Diath. haemorrh. Suppl.* **42**, 21 (1970).
4. B. P. Hilton and J. N. Cumings, *J. Clin. Path.* **24**, 250 (1971).
5. G. V. R. Born, *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.* **259**, 155 (1968).
6. G. V. R. Born, *Exp. Biol. Med.* **3**, 71 (1968).
7. S. Bygdeman, *Acta physiol. scand.* **73**, 28A (1968).
8. S. Bygdeman and Ø. Johnsen, *Acta physiol. scand.* **75**, 129 (1969).
9. R. E. A. Gadd and S. Clayman, *Experientia, Basel* **28**, 719 (1972).
10. G. V. R. Born and J. B. Smith, *Br. J. Pharmac.* **39**, 765 (1970).
11. R. J. Haslam, in *Physiology of Hemostasis and Thrombosis* (Eds. S. A. Johnson and W. H. Seegers), p. 88. Charles C. Thomas, Springfield, Ill. (1967).
12. D. C. B. Mills, I. A. Robb and G. C. K. Roberts, *J. Physiol., Lond.* **195**, 715 (1968).

* W. Barthel, unpublished observation.

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Effects of *O*-2′ and N^6-acyl-substituted cyclic AMP on adipose tissue metabolism *in vitro*

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Cyclic AMP (cAMP) mediates in many of the actions of a variety of hormones[1]. Following beta-adrenergic stimulation, fat cell adenyl cyclase promotes formation of cAMP; this allosteric effector[2] binds to protein kinase which then promotes activation of hormone-sensitive lipase resulting in glycerol and free fatty acid (FFA) production. The dibutyryl derivative of cAMP (dbcAMP) is a well-known potent lipolytic agent in adipose cells incubated *in vitro* (e.g. ref. 4). Its high potency is partly attributable to its lipophyllic nature allowing rapid penetration of fat cell membranes[5], and partly to resistance to phosphodiesterase activity[6]. Solomon *et*