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N-Acetyl groups and analgesia

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The presence of acetyl groups in several analgesic drugs of widespread use raises the question whether acetyl group as a structural entity in these compounds confers any special attributes to these drugs. Some of the very commonly used drugs such as paracetamol (acetaminophen), phenacetin (acetaminophenetidine) and aspirin (acetyl salicylic acid) are all acetylated compounds; in the former two drugs an *N*-acetyl group and in the latter an *O*-acetyl group is present. Heroin, obtained by acetylating both the hydroxy groups of morphine is more euphoria-producing than morphine. A consideration therefore of the importance or influence of these acetyl groups on analgesia appears warranted.

Role of acetyl group in analgesia

In the case of analgesic drugs like phenacetin or paracetamol the *N*-acetyl group is considered to reduce the toxicity of these compounds and thereby enhance their usefulness. Both phenacetin and paracetamol are reported to cause hepatotoxicity possibly through their *N*-hydroxy derivatives. Aspirin is an inhibitor of platelet function and induces markedly prolonged bleeding times in hemophiliacs. The mechanism of action of aspirin (an *O*-acetylated compound) as described in recent work on platelets and sheep vesicular glands [1, 2] suggests that aspirin acetylates the enzyme prostaglandin cyclooxygenase resulting in the formation of an inactive form of the enzyme. The acetylation is irreversible and persists for the life span of the platelet. An irreversible blockade of prostaglandin formation by both aspirin and indomethacin has also been shown [3]. The work of Roth and Siok [4] indicates that the amino group of a serine residue in the purified prostaglandin cyclooxygenase undergoes the acetylation. However more recent work [5] suggests that the hydroxyl group of an internal serine residue in the cyclooxygenase is acetylated by aspirin. It is presumable that this inactivation of the enzyme by aspirin must result in a decreased level of prostaglandin PGG_2. There is however a lack of correlation between enzyme acetylation and analgesic effect. Indomethacin which does not possess acetyl groups can also inhibit the cyclooxygenase and produce analgesia. Evidence for other analgesic acetyl compounds (like paracetamol) acting as acetylating agents of the enzyme is also lacking. It is not conclusively known whether all the pharmacological actions of aspirin can be explained on the basis of the inhibition of prostaglandin synthesis [6]. It has been further suggested that the ability of aspirin like drugs to inhibit the formation of the chemotactic hydroxyfatty acids in neutrophils may also contribute to their antiinflammatory and other pharmacological activity [6].

An N*-acetylated endorphin*

The recent finding [7] in porine pituitary of an *N*-acetylated endorphin which was shown to be an inactive form of this peptide is of great interest. It was inactive in opiate binding assays and in tests for analgesic properties. Amino acid analysis indicated that the $-NH_2$ group of the *N*-terminal tyrosine residue was acetylated in this peptide. It was suggested that the acetylation might be a post-translational modification of the peptide. There was no evidence for the deacetylation of this *N*-acetyl peptide when injected intracerebroventricularly [7]. If this acetylated endorphin were an inactive precursor of active endorphin, then there must obviously be mechanisms for its N-deacetylation to generate the active analgesic peptide.

N*-Deacetylases*

The above mentioned considerations point towards the importance of enzymes which deacetylate *N*-acetylated compounds. *N*-acetyl or *N*-acyl groups occur in a wide variety of naturally occurring compounds and some of the corresponding *N*-deacylases have also been characterized. More relevant to our discussion are the *N*-deacetylases which act on the $-NHCOCH_2R$ group found in amino acids, amines, peptides or aromatic ring structures. *N*-deacetylation of *N*-acetyl amino acids or amines has been shown to be catalysed, by deacylases [8] which have recently been characterized in the kidney and liver [9–11]. Gade and Brown [12] have shown that in bovine liver *N*-acetyl peptides are initially hydrolysed to give rise to *N*-acetyl amino acids which undergo deacetylation. An aromatic *N*-deacylase has been found in liver which can deacetylate several aromatic *N*-acetyl compounds to varying extent [13]. Paracetamol appeared to be more resistant to *N*-deacetylation by this liver enzyme [13].

Aryl acylamidases are enzymes which can *N*-deacetylate a synthetic substrate like *O*-nitroacetanilide. Aryl acylamidases appear to exist in different forms with respect to their sensitivity to inhibition by serotonin. A serotonin sensitive form of this enzyme has been found in brain, erythrocytes and electric eel [14–17]. This enzyme was highly specific for inhibition by serotonin and it was also found to be inhibited by acetylcholine and its analogues [14, 16, 18]. Later studies have shown the association of the serotonin sensitive aryl acylamidase with acetyl cholinesterase [16–18]. A serotonin insensitive form of aryl acylamidase has been noted in the liver [15, 19] and some non neural tissues of the rat [20]. A search for the natural substrates for these aryl acylamidases is of current interest. There is some evidence to suggest that the liver but not the brain aryl acylamidase deacetylates melatonin [21]. A phenacetin *N*-deacetylase found in monkey brain did not appear to be the same as the serotonin sensitive aryl acylamidase [22].

The serotonin sensitivity of brain aryl acylamidase leads to interesting speculations. It is known that the brain depends to a great extent on dietary tryptophan for serotonin [23]. Serotonergic neurons are involved in analgesia and low concentrations of serotonin in the neurons could be associated with high responsiveness to pain and to stimuli in general [24]. If the serotonin sensitive brain aryl acylamidase were to act on an *N*-acetylated analgesic compound then higher levels of serotonin may be expected to preserve and prolong the survival time of such a compound by inhibiting its deacetylation. Whether any naturally occurring *N*-acetyl components responsible for analgesic action would serve as substrates for the serotonin sensitive aryl acylamidase remains to be seen.

Almost 80% of the intracellular population of Ehrlich ascites cell and other mammalian proteins are found to be *N*-acetylated [25, 26] and it has been suggested that this blocking group may protect proteins from proteolytic degradation [27]. If *N*-acetyl endorphin were to be a substrate for a serotonin sensitive *N*-deacetylase then an increased level of serotonin would result in a higher level of the *N*-acetyl endorphin. This acetyl peptide may be less susceptible to proteolytic digestion and thus the ultimate effect of serotonin could be a greater availability of the intact *N*-acetyl endorphin, the peptide precursor of active endorphin, in times of need.

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REFERENCES

1. G. J. Roth, N. Stanford and P. W. Majerus, *Proc. Natn. Acad. Sci., U.S.A.* **72**, 3073 (1975).
2. G. J. Roth, N. Stanford, J. W. Jacobs and P. W. Majerus, *Biochemistry,* **16**, 4244 (1977).
3. W. L. Smith and W. E. M. Lands, *J. biol. Chem.* **246**, 6700 (1971).
4. G. J. Roth and C. J. Siok, *J. biol. Chem.* **253**, 3782 (1978).
5. F. J. Van der Ouderaa, M. Buytenhek, D. H. Nugteren and D. A. Van Dorp, *Eur. J. Biochem.* **109**, 1 (1980).
6. M. I. Siegel, R. T. McConnell, N. A. Porter, J. L. Selph, J. F. Truax, R. Vinegar and P. Cuatrecasas, *Biochem. biophys. Res. Commun.* **92**, 688 (1980).
7. D. G. Smyth, D. E. Massey, S. Zakarian and M. D. A. Finnie, *Nature Lond.* **279**, 252 (1979).
8. J. P. Greenstein and M. Winitz, in *Chemistry of the Amino Acids.* Vol. 2, pp. 1753–1767. Wiley, New York (1961).
9. Y. Endo, *Biochim. biophys. Acta.* **438**, 532 (1976).
10. Y. Endo, *FEBS Lett.* **95**, 281 (1978).
11. Y. Endo, *Biochim. biophys. Acta.* **523**, 207 (1978).
12. W. Gade and J. L. Brown, *J. biol. Chem.* **253**, 5012 (1978).
13. R. H. Nimmo-Smith, *Biochem. J.* **75**, 284 (1960).
14. A. Oommen and A. S. Balasubramanian, *Biochem. Pharmacol.* **26**, 2163 (1977).
15. D. Fujimoto, *Biochem. biophys. Res. Commun.* **61**, 72 (1974).
16. S. T. George and A. S. Balasubramanian, *Eur. J. Biochem.* **111**, 511 (1980).
17. D. Fujimoto, *FEBS Lett.* **71**, 121 (1976).
18. A. Oommen and A. S. Balasubramanian, *Eur. J. Biochem.* **94**, 135 (1979).
19. A. Oommen and A. S. Balasubramanian, *Biochem. Pharmacol.* **27**, 891 (1978).
20. A. Oommen and A. S. Balasubramanian, *Ind. J. Biochem. Biophys.* **16**, 264 (1979).
21. M. A. Rogawski, R. H. Roth and G. K. Aghajanian, *J. Neurochem.* **32**, 1219 (1978).
22. A. Oommen, S. T. George and A. S. Balasubramanian, *Life Sci.* **26**, 2129 (1980).
23. R. J. Wurtman and J. D. Fernstrom, *Biochem. Pharmacol.* **25**, 1691 (1976).
24. R. B. Messing and L. D. Lytle, *Pain* **4**, 1 (1977).
25. J. L. Brown and W. K. Roberts, *J. biol. Chem.* **251**, 1009 (1976).
26. J. L. Brown, *J. biol. Chem.* **254**, 1447 (1978).
27. H. Jornvall, *J. Theor. Biol.* **55**, 1 (1975).