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ACTIVATION OF BRAIN PHENYLETHANOLAMINE-N-METHYLTRANSFERASE BY TRITON-X-100 : TIME RELATED DIFFERENCES IN THE ENZYME ACTIVATION

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SUMMARY: The effect of different concentrations of Triton-X-100 (0.2 to 5 %) on the activity of enzyme phenylethanolamine-N-methyltransferase (PNMT) in the brain and adrenal was studied. The addition of 0.2 % Triton-X-100 to the 0.9 % KCl homogenization media resulted in 180 % activation of the brain PNMT. The similar content of this detergent added to the adrenal PNMT preparation had no marked effect on enzyme activity. Rising Triton-X-100 concentrations from 0.2 % to 5 % resulted in higher activation of brain PNMT activity but the adrenal enzyme remained rather stable. An exposure of 15 minutes of brain PNMT preparation to Triton-X-100 was the optimal interval to evoke the maximal increase in enzyme activity. This activation of brain PNMT by Triton-X-100 was observable up to 24 hours after the addition of the detergent.

INTRODUCTION

Enzyme phenylethanolamine-N-methyltransferase (PNMT) is responsible for N-methylation of noradrenaline to adrenaline (1). It is mainly localized in the adrenal medulla which is the richest source of its activity(1, 2). Small traces of enzyme PNMT were reported to be present in the heart and brain of mammals (3, 4). The presence of PNMT in the brain tissue has received tremendous interest in the recent years mainly due to the role it can play for cerebral circulation and processes of glucose availability (5, 6). Triton-X-100 is widely used for different purification steps of several enzymes of catecholamine synthesis and metabolism (7, 8). However, its use in routine radioisotopic assays of enzyme dopamine beta hydroxylase, tyrosine hydroxylase and monoamine oxidase has received very little attention (7, 8, 9). It was found that low homogenization con-

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centrations of Triton-X-100 activate enzyme tyrosine hydroxylase (10). The regional determinations of different enzymes of catecholamine synthesis and metabolism in brain nuclei dissected by micro-punch technique limit the weight of the tissue to mg fractions (11, 12). The present study shows that the presence of Triton-X-100 in the homogenization media of brain tissue acts differently for the optimal assay of catecholamine enzymes, especially PNMT. The role of different concentrations of Triton-X-100 added to KCl homogenization media upon activity of adrenal and brain PNMT has been compared. The effect of time exposure from 5 minutes to 24 hours on the assay of PNMT utilizing radioactively labelled S-adenosylmethionine-methyl has also been studied.

MATERIALS AND METHODS

Brain and adrenals were excised from albino rats after cervical fraction. The tissues were homogenized in 0.9 % KCl at a temperature of 1° C. Different concentrations of Triton-X-100 were added to the KCl homogenates in separate tubes to achieve a final concentration of 0.2, 0.5, 1 and 5 %. The homogenate represented 5 to 10 mg/ml of protein. PNMT was assayed radioisotopically utilizing S-adenosylmethionine-^{14}C-methyl as a co-factor for the transfer of the methyl group to normetanephrine, employed as the substrate (13). High speed supernatant of brain and adrenal homogenates were used as enzyme preparation (13, 14). We noticed that the fractionation speed has no particular influence on the level of enzyme activity (13, 14). The details of the method have been explained previously. The assay was sensitive enough to detect appreciable amount of ^{14}C-metanephrine transformed after one hour of incubation by 0.25 mg of brain protein. For adrenal PNMT assay only a one tenth diluted enzyme preparation gave high level of activity. The same method was equally suitable for both brain and adrenal PNMT determinations. The end product formed showed linearity up to 2 mg of brain protein and 0.25 mg of adrenal protein respectively. Fig. 1 provides a comparison for brain and adrenal PNMT activity in relation to tissue concentration estimated by our assay.

RESULTS

Fig. 2 illustrates the effects of different concentrations of Triton-X-100 added to the KCl homogenate of brain upon the activity of enzyme

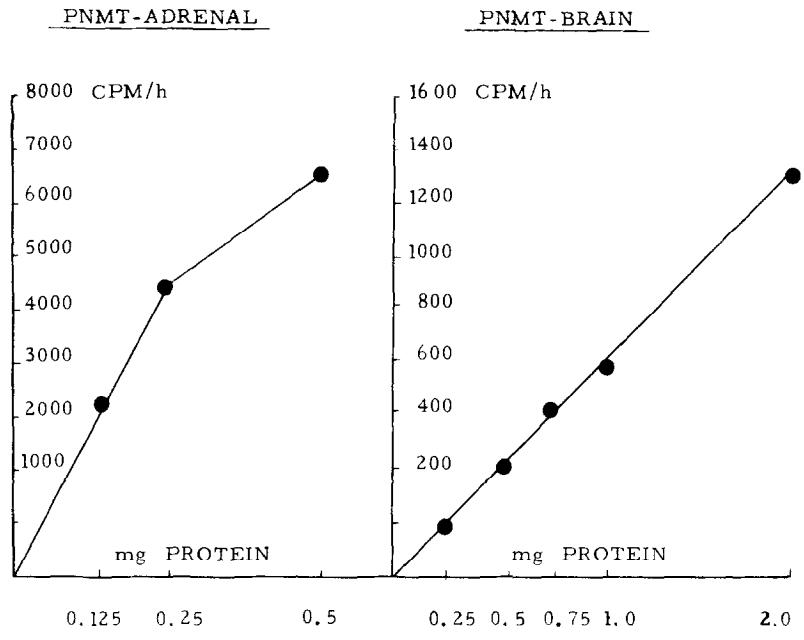

Fig. 1. Relation between tissue concentration and end product extraction in the assay of enzyme PNMT used for brain and adrenal tissue. CPM extracted represent a linear interrelation with pico moles of ^{14}C-metanephrine formed during one hour of incubation at 37° C. Each point represent 6 determinations. Each point showed marked significance from each other for the t test.

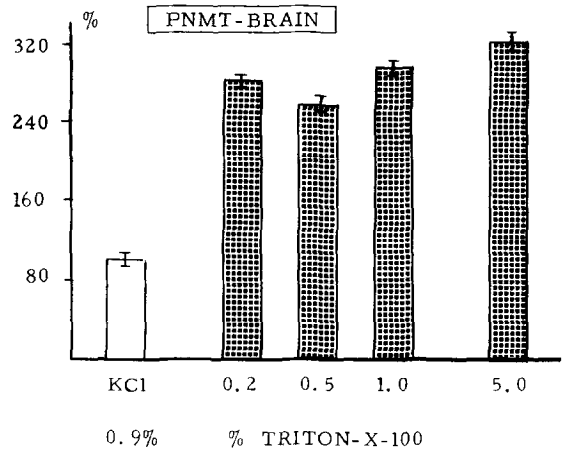

Fig. 2: Influence of Triton-X-100 upon PNMT activity of the brain. All the values are calculated as % of KCl value taken as 100 %. Each group shows a mean of 8 individual determinations. All concentrations of Triton X-100 produced highly significant changes from KCl value ($p < 0.001$).

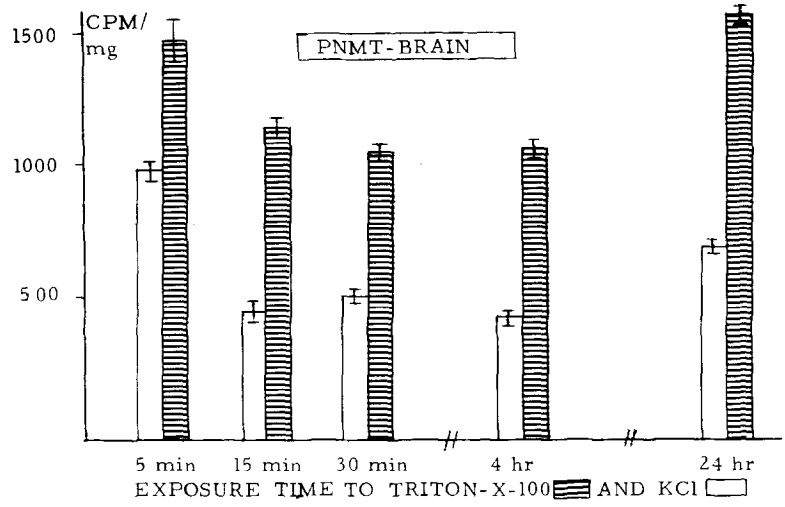

Fig. 3: Time related difference in brain PNMT activity from 5 minutes to 24 hours after exposure to 0.2 % Triton-X-100 (tinted columns) or 0.9 % KCl (transparent columns). All time intervals showed a great degree of PNMT activation right after 5 minutes of Triton-X-100 treatment. All the groups show high statistical significance from KCl controls (p 0.001).

PNMT. PNMT activity increased by 180 % from KCl value when the enzyme preparation contained 0.2 % of Triton-X-100. The increase in concentration of the detergent to 0.5 % and 1 % showed a similar pattern of activity as observed with 0.2 % except that 1 % of Triton-X-100 produced a 200 % rise. When the content of Triton-X-100 was raised to 5 % the activity reached to 220 % higher than the control KCl value.

Fig. 3 shows the influence of brain PNMT exposure to Triton-X-100 (0.2 %) upon maintenance of enzyme activity between 5 minutes to 24 hours. Five minutes of exposure produced 50 % rise in activity. The highest rise was observed after 15 minutes of Triton-X-100 treatment. However, longer treatment of enzyme preparation with this detergent, from 30 minutes to 24 hours all resulted in marked activation of enzyme activity. The enzyme was rather stable if kept at 4° C up to 24 hours in Triton-X-100-KCl media.

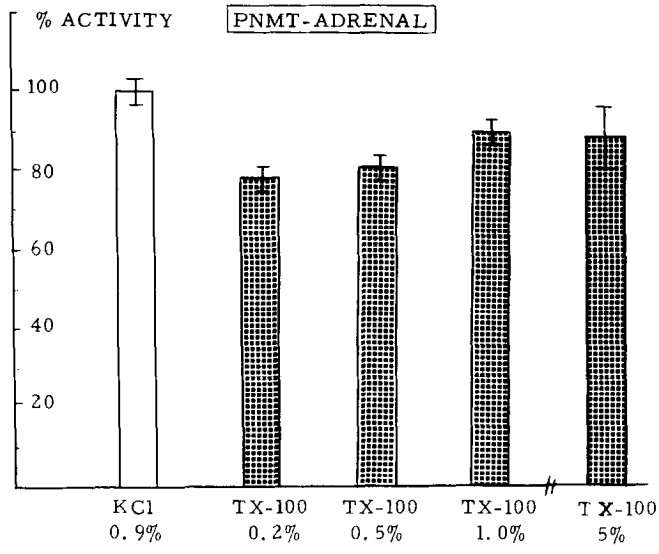

Fig. 4. Effect of Triton X-100 on adrenal PNMT activity as compared with normal KCl level. No significant effect on adrenal enzyme was found with 1% Triton addition. However 0.2 and 0.5 % of this detergent decreased the enzyme activity by 20 and 17 % respectively which were just at the level of probably significant. Each column represents a mean of 6 to 10 different values.

Fig. 4 shows the influence of Triton-X-100 on PNMT activity of the adrenal gland. It is surprizing to observe that the concentrations of this detergent which provoked 200 % increases in brain PNMT, did not have any influence upon adrenal enzyme. Adrenal gland is the main source of PNMT where it is maximally higher than any other region of the body. This experiment was performed to verify the effects of Triton-X-100 on peripheral PNMT which has been described to be different in protein specificity than the cerebral enzyme (15). Since 0.2, 0.5 and 5 % of Triton-X-100 did not evoke any significant effect on adrenal PNMT, no other concentrations were tried.

DISCUSSION

The results presented in this study show that non-ionic detergents such as Triton-X-100 could lead to very high activation of enzyme PNMT

which is the key enzyme for N-methylation of noradrenaline to adrenaline. Such effects of Triton-X-100 were mainly studied on the regulation and maintenance of purified enzyme preparations of dopamine beta hydroxylase (16). It is now a common practice that investigators use a 0.2 to 1 % Triton-X-100 in the homogenization media for the assay of enzyme tyrosine hydroxylase (16), but nothing has been reported on the behavior of other enzymes of catecholamine regulation which are also present in the brain tissue. It appears that Triton-X-100 utilization in the homogenization media could lead to perturbed behavior of different catecholamine regulating enzymes. This can be the case for enzyme monoamine oxidase which has been shown to be inhibited by this detergent (17). The activation of brain PNMT can be mainly attributed to be physical effect since it does not alter adrenal PNMT where it is very high. Brain tissue contains large amounts of lipids and fat that protect complete solubilization of the enzyme and when this detergent is added a maximum response is obtained. This is not the case with adrenal PNMT which is less contaminated with lipids and fat and therefore no marked effect is observed. The present data suggests the utility of Triton-X-100 for assay of PNMT in brain which has a fundamental role in normal brain function. It is suggested that the homogenization media for each particular enzyme of catecholamine regulation should be carefully studied before its final use since certain ions as well as detergents activate one enzyme system whereas they inhibit the other one.

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