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Stimulation of Brain Lipase Activity by Polyamines. Comparison With the Effect of ACTH

J. LE PETIT, O. NOBILI AND J. BOYER[1]

INSERM U.260, Faculté de Médecine, 27, Bd Jean Moulin, 13385 Marseille Cédex 5, France

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LE PETIT, J., O. NOBILI AND J. BOYER. *Stimulation of brain lipase activity by polyamines. Comparison with the effect of ACTH.* PHARMACOL BIOCHEM BEHAV 24(6) 1543–1545, 1986.—Polyamines, as well as ACTH, strongly stimulate at pH 5.75 triacylglycerol lipase (TAGL) activity from rat brain. Whether the activating potency is expressed in terms of molar concentration or amount of positive charges, polyarginine, polylysine, spermine and spermidine exhibit, in this order, decreasing potencies. By contrast to other lipases, heparin (25 μg/ml) inhibits brain TAGL. Polyarginine, polylysine and spermine reverse the heparin-dependent inhibition and further stimulate TAGL activity above basal values; spermidine is much less potent. In the presence of heparin, ACTH has the greatest stimulating effect, being 1.6-fold and 3.3-fold more potent than polyarginine and polylysine, respectively. Taken together, the data suggest that polybasic effectors modify the interaction of TAGL with its substrate, resulting in increased levels of TAGL activity. In the presence of heparin, the enzyme charge density is mandatory for determining the stimulation process. Such cationic interactions appear to be specific of brain TAGL and should be considered in assessing any direct neuro-hormonal role to ACTH or physiological polyamines in brain.

Polyamines ACTH Heparin Brain lipase

ADRENOCORTICOTROPHIC hormone (ACTH) can mediate behavioral responses by direct cerebral action [6], possibly via the direct stimulation of lipase-dependent hydrolysis of fatty esters in brain membranes [1,2]. In rat brain, the stimulatory effect of ACTH and ACTH-related synthetic peptides on triacylglycerol lipase (TAGL) appears to be associated with the residues Lys-Lys-Arg-Arg (or basic substituents) occurring at position 15–18 in the NH_2-terminal sequence [3]. This contrasts with the effect of ACTH in other tissues, viz. adrenal cortex, where stimulation involves the sequence 1–10 NH_2-terminal, which contains a single basic residue [12]. In an attempt to further explore the stimulatory effect in brain, the influence of synthetic as well as naturally-occurring polyamines on TAGL activity from rat brain was investigated, and compared to that of ACTH. The results show that polyamines, like ACTH or ACTH-related peptides, produce at pHs above 5.6, large increases in brain TAGL activity, whether heparin is present or not as TAGL-inhibiting agent. The data confirm the importance of high positive charge density in determining the stimulation process.

METHOD

Lyophilised fractions (P_2) of brain from Wistar rats (200–220 g) were prepared by the method of Cotman [5] as previously described [2]. Prior to assay, the lyophilisate was dissolved in water (40 mg/ml) and the final preparation served as the enzyme source. Unless otherwise stated, TAGL activity was assayed as previously reported [3] using as the substrate a sonicated emulsion (0.25 ml) of tri-[^{3}H]oleoylglycerol (1 mM, about 10^6 cpm) at 37°C. The reaction was initiated by addition of 25 μl of lipase preparation (about 0.25 mg protein) to the medium containing the substrate and when indicated, the cationic effector. The hydrolysis was monitored through the linear release of [^{3}H]oleic acid during the 10-min period of assay [4]. Duplicate assays were reproducible within 8% of oleic acid released. One milliunit (mU) of lipase activity corresponds to the release of one nmol of oleic acid/min. Enzyme activity is expressed in mU/mg protein at 37°C. Protein concentration was estimated by the method of Lowry *et al.* [10].

ACTH-(1–24), referred to as ACTH, was a gift of Ciba-Geigy (France). Trioleoylglycerol, poly-L-arginine hydrochloride (average mol. wt. 44,000), poly-L-lysine hydrobromide (average mol. wt. 13,000), spermine and spermidine were from Sigma Chemical Co. (St Louis, MO). Sodium taurocholate was from Nutrional Biochem. Corp. (Cleveland, OH). Heparin was from Fluka (Buchs, Switzerland).

RESULTS

As shown in Fig. 1, optimal concentrations of polyarginine (7.5×10^{-7} M) or polylysine (2.5×10^{-6} M) caused com-

[1]Requests for reprints should be addressed to Dr. J. Boyer, Hôpital de la Conception, 13385 Marseille Cédex 5, France.

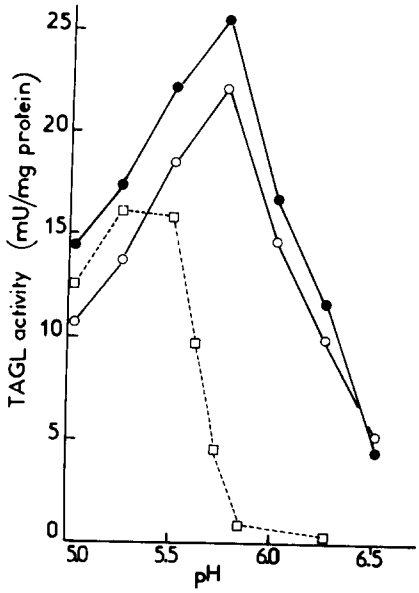

FIG. 1. Effect of pH on TAGL activity from rat brain in the absence (□) and presence of 1.5×10^{-7} M polyarginine (●) or 2×10^{-6} M polylysine (○) in the lipolytic medium. Reactions were started by adding the enzyme (25 μl, about 0.25 mg protein) to the substrate emulsion containing (or not) the polyamine and adjusted to the proper pH. One milliunit (mU) of lipase activity corresponds to the release of one nmol of oleic acid/min at 37°C.

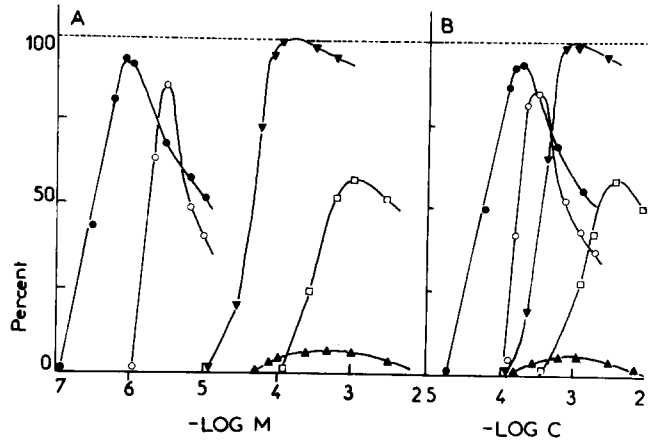

FIG. 2. Log concentration-response curves for the effect of polyarginine (●), polylysine (○), ACTH (▼), spermine (□) and spermidine (▲) on TAGL activity from rat brain assayed at pH 5.75. A: in abscissa, the log of molar concentrations is given; in ordinate, TAGL activity is expressed as percentage of maximal activity with ACTH (22 mU/mg protein) taken as 100%. Each point is the mean of at least two separate determinations in duplicate; activity values agreed within 11%. B: log concentration-response curves as in A except that in abscissa is given for each effector the log of the concentration in positive charges (m-equiv. per ml of lipolytic medium, log C). Symbols and experimental conditions as in A.

parable shifts of the pH optimum for trioleoylglycerol hydrolysis from pH 5 to pH 5.75, as well as comparable increases (about 50%) in maximal reaction rates. This pH shift was quite similar to that resulting from the addition of ACTH to the same lipolytic medium [3]. Reproducible data points could not be obtained with spermine at pH values below 5.5, probably because of the high basic character of this polyamine. We could however estimate that the pH optimum for TAGL activity in the presence of spermine was comprised in the range 5.6–5.8. Lipolytic rates were therefore measured at pH 5.75 with increasing concentrations of polyarginine, polylysine, spermine and spermidine (Fig. 2A). With polyarginine, V increased from zero (at 10^{-7} M) to maximal value at about 7.5×10^{-7} M and then decreased. With polylysine, the activity profile culminated at 2.5×10^{-6} M. Spermine was active at much higher concentration (about 10^{-3} M). Spermidine had only a slight stimulating effect. As shown (Fig. 2A), ACTH had an intermediate potency, with a maximal response at 10^{-4} M. Notably, the V max measured with ACTH was slightly but repeatedly higher than that with any other polyamine. Since the stimulatory effect appeared to be related to the cationic nature of the effectors, reaction rates were plotted against the amounts of positive charges (i.e., basic residues) introduced in the medium with each polycation (Fig. 2B). On this basis, polyarginine again exhibited maximal activating ability whereas polylysine, ACTH and spermine had, in this order, decreasing potencies. The concentration of charges required for each polycation to induce half-maximal response were 7×10^{-5}, 2×10^{-4}, 3.5×10^{-4} and 2×10^{-3} m-equiv./ml for polyarginine, polylysine ACTH and spermine, respectively.

Heparin strongly inhibited TAGL activity. At 5 μg/ml, inhibition averaged 50%, whereas at 25 μg/ml, TAGL was

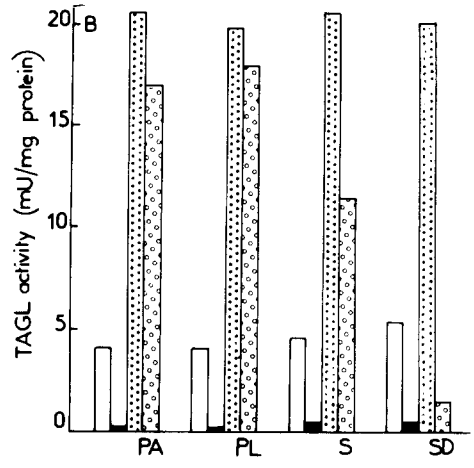

FIG. 3. Comparative effects of ACTH and various polyamines on TAGL activity uniformly inhibited by heparin (25 μg/ml) and measured at pH 5.75. Each group of four columns present the results of concomitant assays of: open column, basal TAGL activity without heparin; solid column, TAGL activity with heparin; column with asterisks, heparin-inhibited TAGL activity values assayed in the presence of a constant concentration of ACTH (5×10^{-5} M); column with circles, heparin-inhibited TAGL activity assayed in the presence of optimal concentration of one of the following polyamines: polyarginine (PA, 2.5×10^{-6} M), polylysine (PL, 7.5×10^{-6} M), spermine (S, 2×10^{-3} M) and spermidine (SD, 10^{-3} M). Data are means of triplicate determinations in a representative experiment. Each set of experiments (four columns) related to PA, PL, S and SD has been carried out on separate days with different preparations of brain lipase. Experiments were replicated at least twice.

inhibited by more than 90%. This heparin effect was differentially antagonized by optimal concentrations of polycations (Fig. 3). Polyarginine and polylysine, at 2.5×10^{-6} M and 7.5×10^{-6} M, respectively, reversed the heparin-dependent inhibition and further stimulated TAGL activity about 4-fold over noninhibited basal values. In the presence of heparin (25 μg/ml) and in terms of positive charges polyarginine had a maximal stimulatory effect at 5.5×10^{-4} m-equiv./ml vs. 1.05×10^{-3} for polylysine; maximal activation by spermine (about 2.5-fold over basal values) occurred at 8×10^{-3} m-equiv./ml. In the presence of heparin, ACTH had the greatest stimulatory potency, being 1.6-fold and 3.3-fold more efficient than polyarginine and polylysine, respectively, with a maximum at 3.5×10^{-4} m-equiv. of positive charges/ml (Fig. 3). The stimulatory effect was not simply due to polycation-dependent increases in ionic strength, since addition of NaCl resulted in up to 60% inhibition of TAGL (data not shown).

DISCUSSION

The stimulatory effect of polyamines on TAGL from rat brain occurs under experimental conditions similar to those required for the stimulatory effect of ACTH on this enzyme [3]. Polyamines cause an alkaline shift of the pH optimum of hydrolysis as well as an increase in maximum TAGL activity. The greater potenices of polyarginine (220 basic residues) and polylysine (140 basic residues) compared with spermine (a tetramine) and spermidine (a triamine) shows that stimulation is related in some way to the basic character of the effectors. This is consonant with previous data on the effects of ACTH and ACTH-related peptides on rat brain TAGL [3]. It is unlikely that stimulation of TAGL by polycations occurs through a cyclic nucleotide-dependent multienzyme cascade: (1) the effect requires the presence of the effector in the catalytic medium; (2) the high concentrations ($>10^{-7}$ M) required for full effect are far above those needed for receptor interaction. These characteristics, although circumstantial, suggest a direct influence of polyamines and ACTH on the enzyme-substrate interaction.

In terms of activity per basic residue, the polycations are not equipotent (Fig. 2B). In addition to their polybasic character, their effect is related in some way to chemical structures and environmental factors. For example, whilst polyarginine shows a potency greater than ACTH under basal conditions, ACTH exhibits the highest activation rate on heparin-inhibited TAGL activity. Such changes preclude any extrapolation to in vivo condition, where structure and environment of the microdomain of TAGL action are unknown. Interestingly, ACTH increases the fluidity of synaptic membranes from rat forebrain [7]. This observation supports our hypothesis that TAGL-dependent lipolysis may modify the lipid matrix of brain membranes and thereby may modulate the activity of membrane-associated enzymes [3].

The inhibitory effect of heparin on brain TAGL contrasts with the effects of this polyanion on other lipases, where it causes stabilizing and/or activating effects as well as cellular release of enzyme, depending on the experimental conditions [11]. Moreover, strongly basic proteins such as polylysine or protamine, which activate the brain enzyme, rather inhibit other tissue lipases [8,9].

The mechanism by which heparin interferes in the present lipolytic system is at present unclear. It should be noted that the absolute activity reversal of brain TAGL by the polycations acting in the presence of heparin brings the enzyme levels back to nearly the same maximal values that are attained with these polycations in heparin-free medium (compare Figs. 1, 2 and 3). But, in the presence of heparin, maximal values are obtained with higher concentrations of polycations: for instance, polyarginine restored maximal TAGL values at 2.5×10^{-6} M, and polylysine at 7.5×10^{-6} M, as compred to 7.5×10^{-7} and 2.5×10^{-6} M, respectively, in the absence of heparin. These observations together suggest that ionic interactions occur between at least the polycationic effectors and negatively-charged heparin, resulting in a modified polycation-dependent stimulatory effect.

Whatever the mechanism, brain TAGL appears to be a lipase endowed with particular properties, among which is its remarkable sensitivity to polybasic agents. Such interaction could be considered as at least one possible mechanism in assessing any direct neuro-hormonal role to ACTH or physiological polyamines.

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