Commentary

Commentary on: 'Effect of purified soluble urokinase receptor on the plasminogen prourokinase activation system' by N. Behrendt and K. Dano, *FEBS Letters*, 393 (1996) 31–36

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The paper entitled 'Effect of purified, soluble urokinase receptor on the plasminogen prourokinase activation system' by N. Behrendt and K. Dano was recently published in *FEBS Letters* [1]. In this paper the authors examined the effect of suPAR on plasminogen activation. Although they were able to confirm the results obtained by our group on the stimulatory effect of suPAR on scuPA activity [2,3], they reached contradictory conclusions i.e. that the stimulatory effect observed by them was due to contamination of suPAR by proteases and that the same was probably also the case in our experiments. These conclusions were based on two independent criteria: (1) the effect of ATF to block stimulation of scuPA activity by suPAR and (2) the concentration dependence of the suPAR effect.

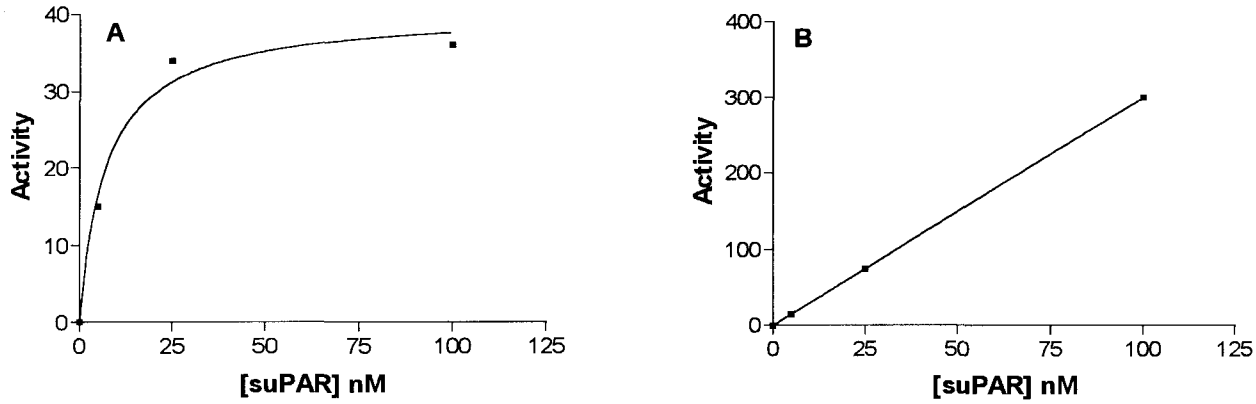

Fig. 1. The effect of suPAR on scuPA-mediated plasminogen activation. A: Initial velocities were calculated from the curves presented in Fig. 3B of the paper by Behrendt and Dano, by determining the slope of the linear portion of each curve. B: The theoretical kinetic pattern that would be obtained if a contaminating protease were present.

As far as the concentration dependence is concerned, the argument is that since increasing concentrations of suPAR above the apparent Kd, still has a stimulatory effect, this effect has to be ascribed to contamination by a protease. This argument is based on a mistaken interpretation of the data presented in Fig. 3B of their paper. From the data in Fig. 1A, which show the initial velocity of the experiments presented in Fig. 3B of the paper by Behrendt and Dano, it can be seen that the effect of suPAR on scuPA activity is dose dependent and saturable. Saturation is already apparent at suPAR concentrations of 25 nM and half maximal stimulation was achieved at suPAR concentrations of 8 nM. The fact that the effect of suPAR is saturable cannot be ascribed to an increase in the concentration of a putative contaminant. If there were a contaminating enzyme present, the kinetic pattern should be as in Fig. 1B of this communication. In other words, the activity of any enzyme is always proportional to its concentration. The experiment presented in Fig. 3B of the original paper should not be an exception, since the enzyme activity would not be limited by the concentration of scuPA, plasminogen or plasmin substrate (as can be seen in the experiment with 100 nM suPAR). The presence of a saturable effect is in accordance with the postulate that the observed stimulatory effect is the result of binding of ligand to receptor (scuPA to suPAR).

The fact that dose dependence of suPAR can be observed even at concentrations that are above the apparent K_d, does not exclude the possibility that suPAR exerts its effect through interaction with scuPA. The K_d is determined by measuring the binding of scuPA to uPAR in a purified system and in the absence of plasmin(ogen) and plasmin substrate. Indeed, Spect-PL, at a concentration of 0.5 mM, is a competitive inhibitor of scuPA binding to uPAR (Higazi et al., unpublished observations). In addition, the fact that scuPA binds to suPAR at several epitopes distributed among the three domains of suPAR, with affinities significantly lower than the published K_d [4], supports the possibility that some interactions between scuPA and suPAR (that are important for the stimulation of scuPA) may take place only at high concentrations and will only be present in equilibrium.

The second criterion was the blocking effect of ATF (Fig.

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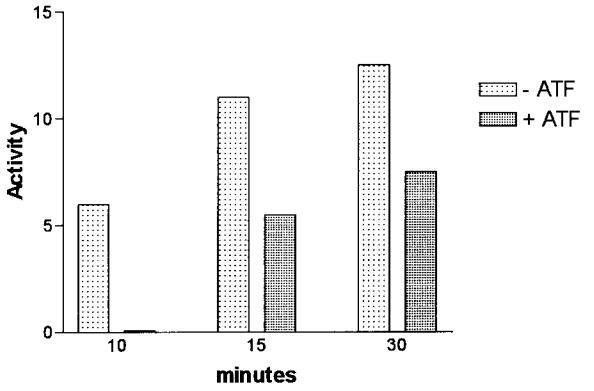

Fig. 2. The effect of ATF on suPAR-mediated stimulation. The data were taken from Fig. 2B of the paper by Behrendt and Dano.

2B of the original paper) and the presentation of the same data in Fig. 2 of the present communication. The authors state that ATF did not affect the stimulation of the cascade resulting from the addition of suPAR. Looking carefully at Fig. 2B it is clear that at 10 min of incubation ATF totally abolished the stimulatory effect of suPAR and at 15 min there was still 50% inhibition. It is indeed to be expected that, under certain conditions, an inhibitory effect would be present only at early time points. The time-dependent decrease of the inhibitory effect of ATF is caused by the gradual activation of scuPA by the generated plasmin. The occurrence of such an effect is widely accepted by investigators, including the authors of this paper.

On the other hand, the data presented by Behrendt and Dano are apparently contradictory to previous results obtained by the same group [5] and by Ellis [6] where no stimulation of scuPA plasminogen activation by suPAR was found, although the putative contaminant should also have been present in their suPAR preparations. We recently found that the basis for the discrepancy between our results and those of Ellis was the use of different plasmin substrates. SuPAR has a stimulatory effect on scuPA activity when the plasmin substrate Spect-PL (American Diagnostics) is used, whereas an inhibitory effect was obtained with S-2251 (Chromgenics) [7]. The fact that the stimulatory effect of suPAR on scuPA activity depends on the plasmin substrate used argues against the possibility that a contaminating protease is responsible for the stimulation obtained in the presence of suPAR.

Finally, the fact that the stimulatory effect of suPAR on scuPA activity depends on the experimental conditions, does not preclude its existence and suggests that we are dealing with a sensitive and sophisticated system, capable of responding to environmental changes.

References

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