Diabetologia 10, 53—59 (1974)

Dynamics of Insulin Release and Microtubular-Microfilamentous System

II. Effect of Vincristine

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Received: May 30, 1973, and in revised form: October 8, 1973

Summary. In order to document the participation of microtubules in the dynamics of insulin release, the secretory response of the isolated perfused rat pancreas was measured after various times of exposure to vincristine (2.10^{-5}M). After a short exposure time (25 min), both phases of glucose-induced insulin release were increased. After longer pretreatment (60 min), this facilitating effect disappeared and a slight, insignificant reduction of both phases of the secretory response to glucose was observed. A still longer exposure time (120 min) provoked a more marked and significant inhibition of the early and late phases of insulin release. The same enhancing effect after short pretreatment with vincristine was noticed when gliclazide was used as the insulinotropic agent. The ultrastructural studies indicated a progressive disappearance of microtubules concomitantly with an increase in number and size of vincristine-induced paracrystalline deposits. These findings suggest that microtubules indeed participate in the dynamics of insulin release, a reduction of both phases of insulin secretion being caused by an extended disruption of the microtubular apparatus, whereas a more limited disturbance of the microtubular system appears to be associated with facilitated insulin release in response to either glucose or sulfonylurea.

Key words: Insulin secretion, pancreas perfusion, glucose, gliclazide, microtubules, microfilaments, vincristine.

The participation of a microtubular-microfilamentous system in insulin release has been documented by studies on the effect of mitotic-spindle inhibitors (colchicine, vincristine) [1, 2, 3] and a microfilament inhibitor (cytochalasin B) [4, 5] upon insulin secretion *in vitro*.

We have recently reported that cytochalasin B, by disrupting the cell web, enhances both the early and late phases of glucose-induced insulin secretion by the isolated perfused rat pancreas [6], the facilitating effect being observed after both short and prolonged exposure to the mould metabolite.

It remained to be investigated whether the microtubular component of the translocator-releasing system [7] is also actively involved in both phases of insulin secretion. For this purpose, we have here examined the effect of vincristine, a mitotic-spindle inhibitor, upon glucose- and sulfonylurea-induced insulin release by the isolated perfused rat pancreas.

Materials and Methods

1. Perfusion Technique

The detailed description of the perfusion technique was given in our earlier publication [6]. Briefly, the pancreas was dissected according to a technique described elsewhere [8]. Fully fed male albino rats (200 to 300 g body weight) were used as pancreas and blood donors. The perfusate consisted in a bicarbonate-buffered medium containing 4% (w/v) bovine albumin (fraction V, Sigma Chemical Co, St. Louis, Mo.) and 10% (v/v) heparinized rat blood. The perfusate was equilibrated against a mixture of O_2 (95%) and CO_2 (5%), and circulated through the perfusion unit for 10—20 min at 37°C before introducing the isolated pancreas (time zero). Thereafter and for the rest of the experiment no recirculation was allowed to occur, the venous effluent being either discarded or collected over individual periods of one min each. The flow rate through the pancreas was maintained constant in all experiments at 2.0 ml/min. In the experiments carried out in the presence of vincristine sulfate (Oncovin, Eli Lilly and Co, Benelux), this drug was added to the perfusate at a final concentration of 2.10^{-5}M. In order to raise the glucose concentration, or to add gliclazide (S852, Laboratories Servier, Paris, France) small amounts of bicarbonate-buffered solution containing these stimulatory agents were administered into the arterial canula by mean of a Braun pump (Braun, Melsungen, Germany), at a flow rate of 0.05 ml/min, achieving a final concentration of glucose of 3.0 mg/ml or gliclazide of 0.02 mg/ml. The control experiments and those performed with vincristine were carried out on different rats, but usually on the same day, 2 to 8 individual experiments being performed each day.

The glucose concentration of the initial perfusate amounted to 0.3 mg/ml. The arterial pressure, oxygen tension, arterial venous difference in pO_2 and pH of the perfusate were all maintained within the normal physiological range, no obvious influence of vincristine upon these parameters being noticed.

2. Chemical Determinations

The samples of venous blood were processed, as described in detail in our earlier publication [6].

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Glucose was measured by a photoelectric method [9] adapted to the Technicon Auto-Analyser. Insulin was measured by radioimmunoassay [10].

3. Ultrastructural Studies

The preparation of pancreatic tissue for ultrastructural studies has been previously described [6]. Briefly, the pancreas was perfused with phosphate-buffered glutaraldehyde. Pieces of the tail of the pancreas were post-fixed with phosphate-buffered OsO_4, dehydrated in alcohol and embedded in Epon. The whole fixation and dehydration procedure was performed at room temperature. Thin sections of islets, stained with uranyl acetate and lead citrate, were examined with a Philips 300 electron microscope.

For certain experimental conditions, a quantitative study was performed in 10 islets in order both to estimate the number of microtubules in B-cells and to determine the volume density of vincristine-induced paracrystalline deposits. For each islet, the number of microtubular profiles, excluding transverse sections, was counted in 5 randomly taken micrographs, representing a total cytoplasmic area of approximately 80 μ^2. The volume density of paracrystalline deposits in the cytoplasm of B-cells was determined by point counting, according to the stereological principles for morphometry [11].

4. Presentation of Results

In the figures, the mean values ($\pm$SEM) for the rate of insulin secretion, at each time interval and under any given experimental condition, are always expressed in per cent of the mean total amount of insulin release over the entire period of stimulation in the corresponding group of control experiments. Likewise, in order to quantify the effect of vincristine upon the early and late phases of insulin secretion, respectively, the total mean amount of insulin released in the control experiments over the 6 first min of stimulation (early phase) and the ensuing 9 min (late phase) were each considered as the 100% reference value. The statistical analysis is always based on a group comparison between control and experimental data, using Student's t-test.

Results

1. Influence of the Length of the Prestimulatory Period on the Secretory Response of the Pancreas

As shown in Table 1, the rate of insulin secretion in response to glucose was higher when the prestimulatory period was prolonged from 25 min up to 60 or 120 min. The magnitude of the pancreatic secretory response to glucose at the 120 min was not significantly different, whether or not a first stimulus had been applied from the 25th to 40th min. These data confirm previous findings [12]. The possible role of a time-related recovery of the pancreas after the surgical stress or of a progressive depletion of the local pancreatic stores of catecholamines, which might influence the magnitude of the insulin response [13, 14, 15], remains to be evaluated.

Table 1. *Secretory response to a single glucose stimulation applied at different times during the perfusion*

Period of glucose stimulation (min)	Insulin output (per cent of control)	n	P
25→ 34	100± 6	25	
60→ 69	170±18	8	<0.001
120→129	211±18	10	<0.001
120→129*	185±31	5	<0.001

Mean values ($\pm$SEM) for total insulin output over 9 min of stimulation are expressed in per cent of the mean value recorded between the 25th and 34th min, and are shown together with the number of individual determinations (n) and the statistical significance (P) of differences between the earliest and later values. In one group of experiments (*), the pancreas had been exposed to a first glucose load from the 25th to 40th min.

2. Effects of Vincristine upon Glucose-Induced Insulin Release

As shown in Fig. 1, after 25 min of exposure to vincristine (2.10^{-5}M), both phases of glucose-induced insulin release were enhanced. The initial phase, estimated over a period of 6 min after the addition of glucose, corresponded to a total insulin release of $164 \pm 12\%$ ($n=11$) in the presence of vincristine and $100 \pm 8\%$ ($n=16$) in its absence ($p<0.001$), respectively. The secretory rates during the late phase were also significantly higher ($p<0.001$) in the presence of vincristine ($195 \pm 19\%$; $n=11$) than in its absence ($100 \pm 10\%$; $n=16$).

When the exposure time to vincristine was prolonged up to 60 min no enhancement of glucose-induced insulin release was (Fig. 2). On the contrary, a slight but insignificant inhibition of both the early and late phases was observed. This inhibition of the initial and late phases became more marked when the exposure time to vincristine was lengthened to 120 min. The early phase corresponded to a total insulin release of $58 \pm 3\%$ ($n=5$) in the presence of vincristine and $100 \pm 8\%$ ($n=10$) in its absence ($p<0.001$). The insulin secretion rate during the second phase was lowered to $60 \pm 8\%$ ($n=5$) ($p<0.05$) in the presence of vincristine, while the control values averaged $100 \pm 11\%$ ($n=10$) (Fig. 3).

3. Effect of Vincristine upon Sulfonylurea-Induced Insulin Release

As the facilitation of insulin release observed after a short period of exposure to vincristine came to us as an unexpected finding, we have also investigated the effect of such a short pretreatment with vincristine upon the secretory response of the pancreas to sulfonylurea. These experiments were performed in the presence of a low glucose concentration (approximately 0.3 mg/

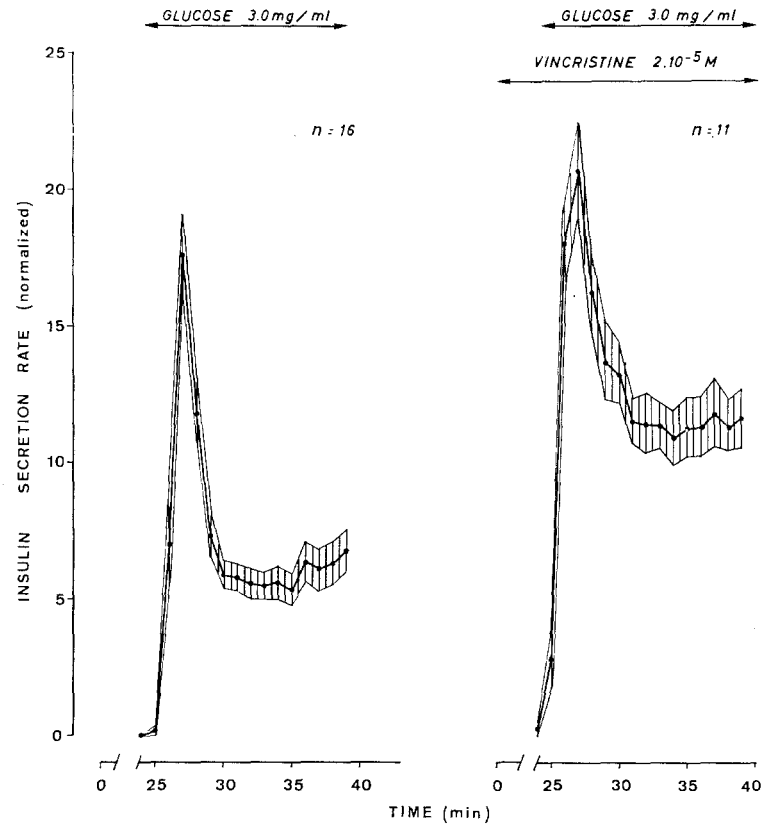

Fig. 1. Effect of vincristine (2.10^{-5}M) on glucose-induced insulin after a short exposure time (25 min) to the mitotic-spindle inhibitor. Mean values ($\pm$ SEM) are expressed in per cent of the mean value for total insulin output in control experiments, and are shown together with the number of individual experiments in each group (n)

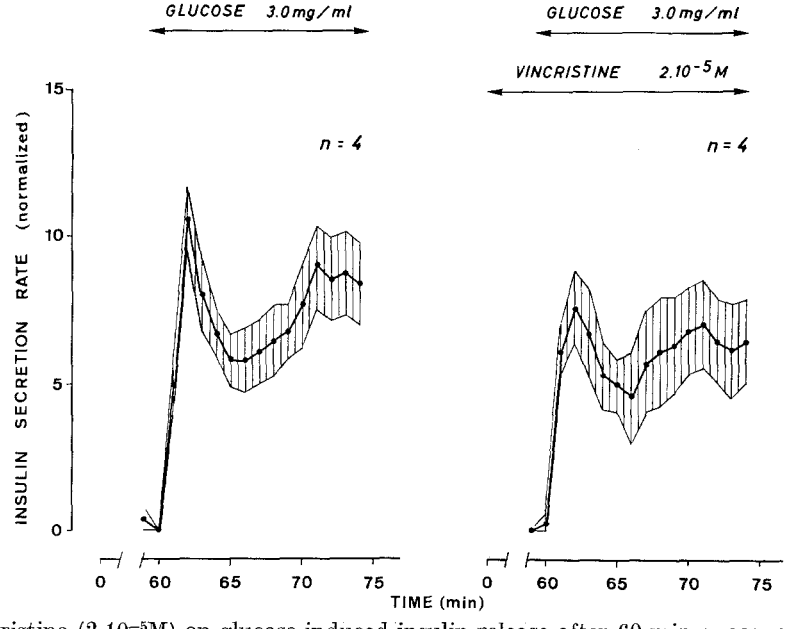

Fig. 2. Effect of vincristine (2.10^{-5}M) on glucose-induced insulin release after 60 min exposure to the mitotic-spindle inhibitor. Same presentation as in Fig. 1

ml). As illustrated in Fig. 4, the early secretory response to gliclazide was also markedly increased after exposure to vincristine, averaging $211 \pm 20\%$ ($n = 11$) as distinct from $100 \pm 9\%$ ($n = 17$) in the control experiments ($p < 0.005$). Vincristine, however, failed to significantly affect the much lower insulin output observed after the initial secretory peak in the presence of gliclazide.

4. Morphological Findings

The ultrastructural appearance of B-cells in control pancreas was comparable to that previously described [6, 16]. Microtubules were found scattered throughout the cytoplasm, where they could be seen in close relationship to various organelles. They were often prominent in the ectoplasmic and Golgi areas.

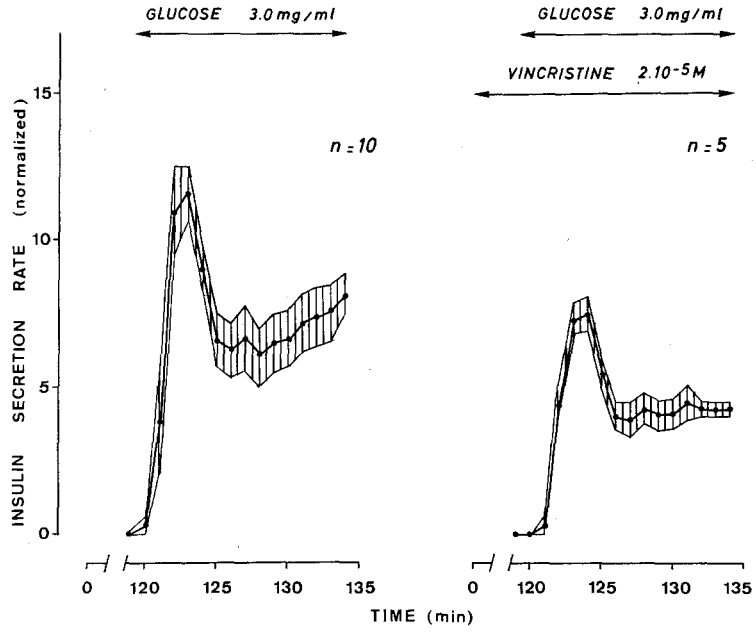

Fig. 3. Effect of vincristine (2.10^{-5}M) on glucose-induced insulin release after 120 min exposure to the mitotic-spindle inhibitor. Same presentation as in Fig. 1

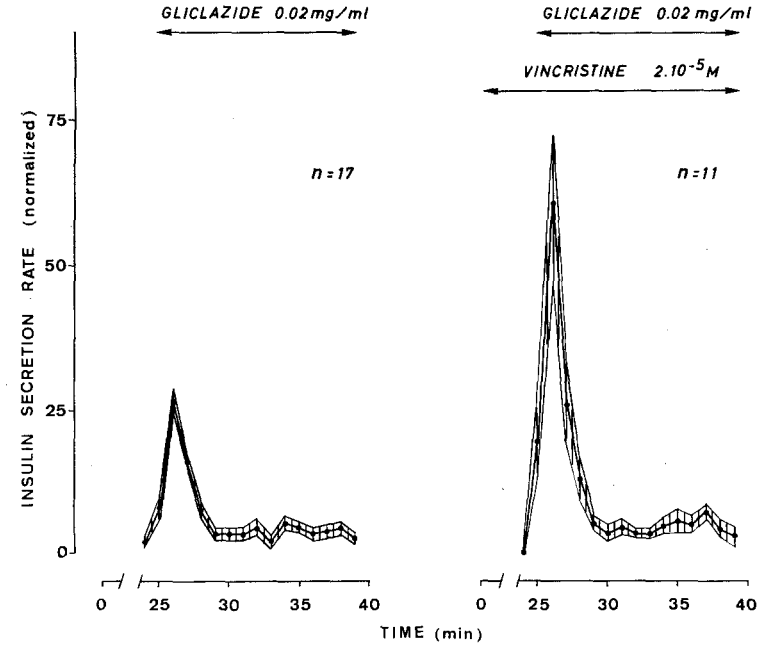

Fig. 4. Effect of vincristine (2.10^{-5}M) on gliclazide-induced insulin release after 25 min exposure to the mitotic-spindle inhibitor. Same presentation as in Fig. 1

In B-cells exposed to vincristine for 25 to 30 min, a few typical paracrystalline deposits were already present. Concomitantly, fewer microtubular profiles were seen than in control pancreases (Fig. 5). After 2 h of exposition to the alkaloid, most of the B-cells exhibited large paracrystalline deposits, whilst microtubules were exceedingly rare (Fig. 6). Thus, the increase in number and size of vincristine-induced deposits paralleled the disappearance of the microtubular profiles (Table 2). No preferential topographical location of the crystals was noted at either time. No obvious effect of vincristine upon the cell web [5] was apparent.

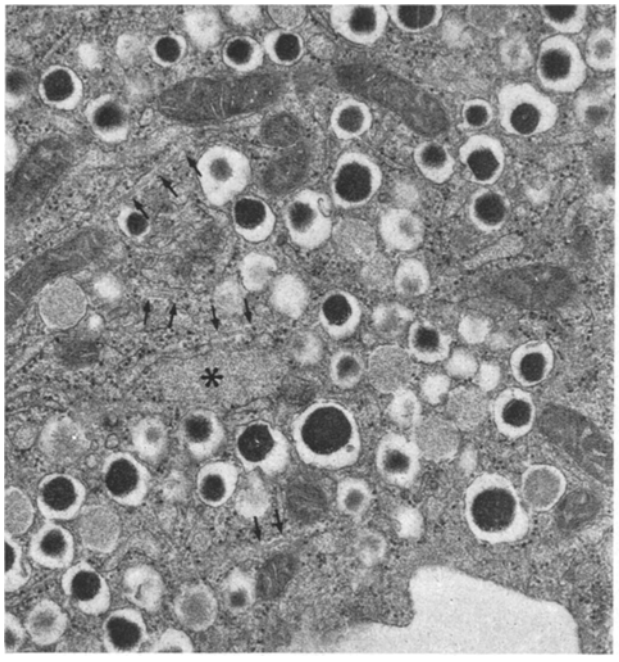

Fig. 5. Pancreas exposed for 30 min to vincristine (2.10^{-5}M). Gliclazide (0.02 mg/ml) was administered from the 25th to 30th min. Microtubular profiles (arrows) and paracrystalline deposit (asterisk) are seen within the same B-cell (magnification × 20.000)

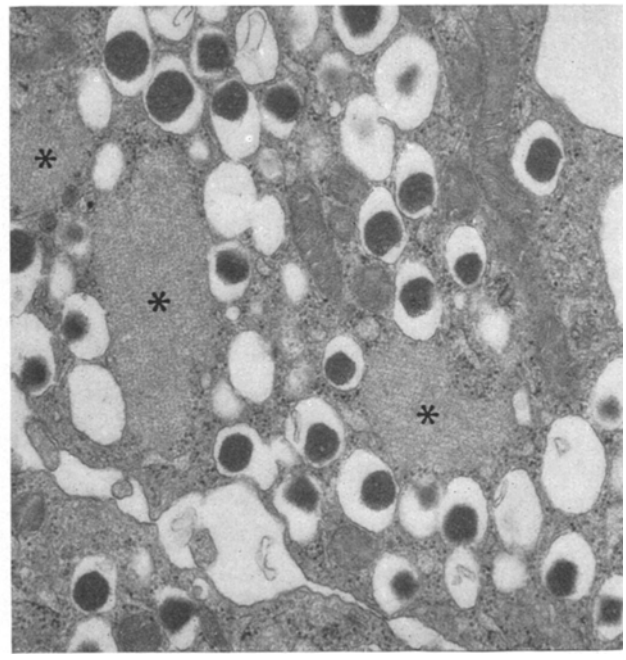

Fig. 6. Pancreas exposed for 135 min to vincristine (2.10^{-5}M). Glucose (3.0 mg/ml) was administered from the 120th to 135th min. Portion of a B-cell exhibiting paracrystalline deposits of varying size (asterisks) (magnification × 20.000)

Table 2. *Effect of vincristine upon microtubules in B-cells*

Exposure time to vincristine	Microtubules frequency	Paracrystalline deposits Volume density (10^{-3})
Nil	47.3±3.1	—
25 to 30 min	16.5±0.9	7.9±1.4
135 min	4.2±0.6	18.7±1.5

Mean values (± SEM) refer to the frequency of microtubular profiles and the volume density of paracrystalline deposits in the cytoplasm of B-cells from control and vincristine-treated pancreases. Ten individual determinations were performed in each group (see Materials and Methods). All differences between mean values were highly significant ($p < 0.0005$).

Discussion

1. The Late Effect of Vincristine

The changes in glucose-induced insulin release observed after a long exposure to vincristine concur with the data obtained with isolated islets [3]: the total amount of insulin released in response to glucose over the first 90 min of incubation was not significantly affected by vincristine, whereas the total output between the 90th and 180th min was significantly decreased, the vincristine-induced reduction during the second period of incubation being of the same order of magnitude as that observed at the 120th min of perfusion. Our findings are also compatible with a recent report [17] indicating that the secretory response of perifused islets to glucose is impaired after 81 min pretreatment by either vinblastine (10^{-4}M) or colchicine (10^{-3}M). In all these studies, the prolonged exposure to mitotic-spindle inhibitors, which was required to cause significant inhibition of insulin release, probably reflects on the time-related disappearance of microtubules. Indeed, the comparison between our functional and ultrastructural findings suggest that an extended disruption of microtubules, such as that observed after 120 min exposure to vincristine, is required to impair the controlled migration of secretory granules towards the cell membrane. Even so, it should be underlined that only a partial reduction of insulin release was observed (Fig. 3). It remains possible, therefore, that

the release of insulin partially occurs in a manner which is not dependent on the integrity of the microtubular system.

2. The Early Effect of Vincristine

After a short period of exposure to vincristine, both phases of glucose-induced insulin release were enhanced (Fig. 1). The same facilitation was observed when colchicine was used instead of vincristine as the mitotic-spindle inhibitor [18] and when gliclazide was used instead of glucose as the insulinotropic agent (Fig. 4). Since this unexpected facilitation of insulin release was observed at a time when the B-cell already contained a few typical paracrystalline deposits, it is likely, though not certain, that the enhancing effect results from the interaction of vincristine with some component of the translocator-releasing system [7]. For instance, we are wondering whether the facilitation of insulin release, which is reminiscent of the facilitating action of cytochalasin B [6], might not result from some kind of functional alteration of the microtubular-microfilamentous system, taken as a whole. Such an alteration could result in a disoriented intracellular movement of secretory granules. Alternatively, the microfilamentous cell web, which is normally anchored to microtubules, might require the overall integrity of the microtubular apparatus in order to duly exert its control upon the access of secretory granules to the cell membrane. However, on the basis of the present results, we cannot rule out the possibility that the facilitating effect of vincristine is secondary to an event taking place outside the B-cell. For instance, glucagon release is known to be enhanced in the presence of mitotic spindle-inhibitors [19] and this, in turn, could enhance insulin release.

3. Conclusion

In summary, the present study suggests (i) that the microtubular system indeed participates in the dynamics of insulin release by the B-cell, extensive disruption of microtubules being associated with a reduction of both the initial and later phase of insulin secretion; and (ii) that a limited perturbation of some component of the vincristine-sensitive system might, possibly, by altering the functional interaction of microtubules and microfilaments, facilitate insulin release in response to either glucose or sulfonylurea.

Acknowledgements: The authors are indebted to C. Van Beuren, A. Tinant and M. Sidler-Ansermet for skillful technical assistance. This work was supported in part by grant 1180 from the Fonds voor Geneeskundig Wetenschappelijk Onderzoek (Brussels, Belgium), grant 20001 from the Fonds de la Recherche Scientifique Médicale (Brussels, Belgium), grants 3.299.70, 3.553.71, 3.8080.72 and 3.384.70 SR from the Fonds National de la Recherche Scientifique (Bern, Switzerland), the Contract of the Ministère de la Politique Scientifique (Belgium) within the framework of the Association Euratom-Universities of Brussels and Pisa, and a grant-in-aid from the Laboratoires Servier (Paris, France).

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