Effect of Segontin and Reserpine on Isolated Medullary Granules

In a previous paper[1] we have shown that tyramine releases catecholamines but not ATP from isolated medullary granules, and that the released catecholamines are almost quantitatively replaced by the uptake of equimolar amounts of tyramine. Segontin [N-(3′-phenylpropyl-(2′))-1,1-diphenylpropyl-(3)-amine], a potent dilator of the coronaries, causes, *in vitro* as well as *in vivo*, a release of catecholamines from the medullary granules[2]. In order to determine whether segontin acts like tyramine by displacing catecholamines from their binding sites we investigated the release of catecholamines and ATP by segontin and its uptake into the chromaffin granules. For comparison, experiments with reserpine were undertaken, as it is known that this compound also releases catecholamines from medullary granules *in vivo* and *in vitro*[3,4].

The chromaffin granules were prepared from suprarenal medulla of cattle as previously described[5] and suspended in sucrose–phosphate buffer *p*H 6·8. The samples (7 ml.) were incubated for 60 min at 37° C under shaking. The granules of the incubated samples as well as of the non-incubated controls were sedimented by centrifugation (15 min, 12,000*g*, in stainless steel tubes), washed once with 3 ml. sucrose–phosphate buffer and sedimented with 12,000*g*. The granules were then suspended in 5 ml. saline; 4 ml. of this suspension were extracted with 0·15 ml. concentrated perchloric acid and centrifuged. The catecholamines and ATP were determined in the supernatant, the former by the method of v. Euler and Hamberg[6], the latter by the method of Strehler and Totter[7]. In order to measure the uptake of ^{14}C-segontin, 1 ml. of the granular suspension was dried at 60° C and the residue dissolved in 1 ml. hyamine hydroxide at 40° C. After cooling, 10 ml. scintillator (4 g PPO and 100 mg POPOP in 1,000 ml. toluene) were added and the radioactivity was determined in a Packard 314*X* liquid scintillation spectrometer. An internal standard (^{14}C-toluene) was used to correct for quenching and efficiency of counting.

Table 1. Uptake of Segontin by Isolated Chromaffin Granules and its Effect on the Release of Catecholamines (CA)

Segontin (μM/sample)	Segontin uptake (μM)	Release of catecholamines (μM)	(%)	CA release/ Seg. uptake
0·165	0·060	1·39	48	23·1
0·280	0·098	2·27	78	23·1
0·476	0·124	2·92	100	23·5

The samples contain in 7 ml.: 2 ml. granules from 140 mg medullary tissue, 0·1 ml. segontin, 0·4 ml. ^{14}C-segontin (9·6 mμM; specific activity 5 μc./2·4 μM) and 4·5 ml. sucrose-phosphate buffer, *p*H 6·8. Incubation: 60 min at 37° C.

During incubation of isolated chromaffin granules at 37° C, catecholamines and ATP are released spontaneously to the same degree[1,6]. The addition of either 0·3 μM segontin or 1 μM reserpine per sample (Fig. 1) proportionally increases the release of catecholamines and ATP so that the original molar ratio, amine/ATP of 4 : 1, remains unchanged. Table 1 shows that during incubation with ^{14}C-segontin the uptake of segontin, as well as the release of catecholamines, depends on the amount of segontin added to the samples. It is interesting to note that the ability of the granules to take up segontin is limited, since after addition of doses of segontin higher than necessary to deplete the granules completely (0·476 μM/sample) no more segontin is taken up; consequently the molar ratio, catecholamine release/segontin uptake, remains constant and is about 23. In a further 14 experiments with various doses of segontin, and therefore various releasing effects (20–100 per cent), the molar ratio, catecholamine release/segontin uptake, was 20·5 (standard deviation, 5·26), that is, uptake of segontin is proportional to the release of catecholamines although 20 times smaller. The determination of the uptake of segontin and of the release of catecholamines in the non-incubated samples indicated that segontin is already taken up from the granules during centrifugation (15 min, 0° C) while the catecholamines are released only during the following incubation at 37° C. Tyramine, on the other hand, is exclusively taken up at the same time as it releases catecholamines. Therefore, at 0° C no uptake of tyramine and no release of catecholamines take place.

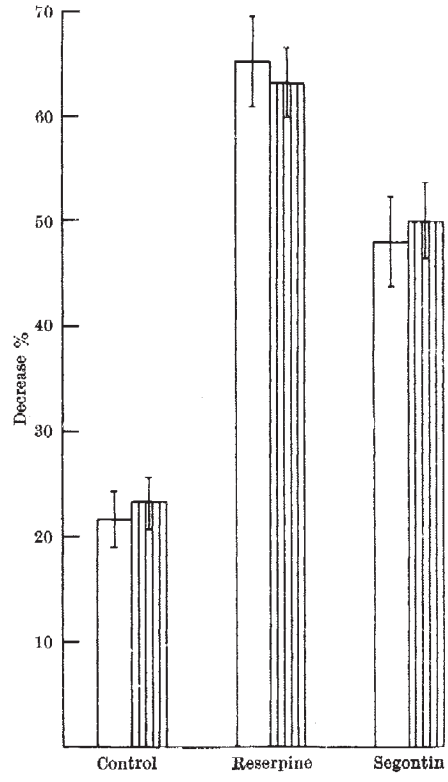

Fig. 1. Release of catecholamines (white) and ATP (hatched) from isolated granules by lyophilized reserpine phosphate (1 μM/sample) and segontin lactate (0·3 μM/sample). The samples contain in 7 ml.: 2 ml. granules from 200 mg medullary tissue, 0·1 ml. segontin or reserpine and 4·9 ml. sucrose-phosphate buffer *p*H 6·8. Incubation: 60 min at 37° C. Means of 4 experiments and their standard deviations

From these results it is obvious that in contrast to tyramine both segontin and reserpine cause, *in vitro*, in addition to the release of catecholamines a proportionate release of ATP. The uptake of segontin, as well as the release of catecholamines, depends on the dose of segontin added. Whereas in the case of tyramine the released catecholamines are replaced by the uptake of equimolar amounts of tyramine, the uptake of 1 mole of segontin is followed by the release of about 20 moles of catecholamines. It can therefore be concluded that the mechanisms of action of tyramine and segontin are different.

We thank Farbwerke Hoechst and Ciba Basle for the supply of labelled segontin lactate and lyophilized serpasil phosphate (reserpine) respectively. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

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