The temperature dependence of [8-^{3}H]adenosine labelling of the perfused bovine and canine adrenal gland

[8-^{3}H]Adenosine can be taken up, phosphorylated, stored and released from the isolated perfused adrenal gland by various secretagogues using a perfusion medium with a temperature of 37° (Stevens, Robinson & others, 1972, 1975). A number of investigators (Douglas & Rubin, 1961; Robinson, 1967; Rubin & Jaanus, 1967; Trifaró, Poisner & Douglas, 1967; Rubin & Miele, 1968; Kovacic & Robinson, 1970) have perfused and stimulated adrenal glands at room temperature (22–25°) with no apparent loss in the ability of the gland to release catecholamines. However, initial attempts, carried out in this laboratory, to label bovine or dog adrenal glands with [8-^{3}H]adenosine perfused at room temperature were unsuccessful. We have therefore investigated the temperature-dependence of storage and release of [8-^{3}H]adenosine by the adrenal gland. Bovine and dog adrenal glands were perfused at 25 and 37° and experiments performed as previously described (Robinson, 1966; Stevens & others, 1972, 1975). Bovine or dog adrenal glands were perfused either at 37° or 25° for from 30 min to 1 h, and then for an additional 30 min with medium containing a total of 100 μCi (canine) or 200 μCi (bovine) of [8-^{3}H]adenosine. Finally the glands were perfused for 30 min with medium alone before stimulation with carbachol. The medium used had the following composition (mM): NaCl, 133; KCl, 4·75; $CaCl_2$, 1·9; KH_2PO_4, 1·18; $MgSO_4$, 1·2; $NaHCO_3$, 25; glucose, 10 and was equilibrated with a mixture of 5% CO_2 in oxygen.

The glands were stimulated with 10 or 100 μg of carbachol and samples of the perfusate were taken both before and after drug stimulation and analysed for cate-

Table 1. *Effect of temperature on the release of catecholamines and radioactivity from isolated perfused bovine and canine adrenal glands.* [8-^{3}H]Adenosine was administered 30 min to 1 h before stimulation. Bovine adrenals were stimulated with 100 μg and canine adrenals with 10 μg of carbachol.

Schedule (post-drug output)	Percent increases above prestimulation concentration 37° Radioactivity	37° Catecholamines	25° Radioactivity	25° Catecholamines
Bovine*				
0–30 s	106 s.d. 8	998 s.d. 58	no increase	653 s.d. 358
30–60 s	125 s.d. 24	1185 s.d. 303	no increase	990 s.d. 353
60–90 s	110 s.d. 38	1132 s.d. 308	no increase	1061 s.d. 196
	(n = 2)		(n = 2)	
Canine†				
0–5 min	207 s.d. 67	819 s.d. 1077	no increase	7700 s.d. 5939
5–10 min	no increase	127 s.d. 117	no increase	1000 s.d. 141
10–15 min	no increase	no increase	no increase	no increase
	(n = 3)		(n = 2)	

* Values presented are for collections of the perfusates during a 30 s interval post drug. Values for a 30 s interval before drug administration are as follows: at 37° 12 s.d. 2 μg (n = 2) catecholamines; 131 s.d. 6 × 10^3 d min^{-1} (n = 2) radioactivity; at 25° 25 s.d. 14 μg (n = 2) catecholamines; 33 s.d. 13 × 10^3 d min^{-1} (n = 2) radioactivity.

† Values presented are for collections of the perfusates during a 5 min interval post drug. Values for a 30 s interval before drug administration are as follows: at 37° 38 s.d. 22 × 10^3 d min^{-1} (n = 3) radioactivity; 0·87 s.d. 0·89 μg (n = 3) catecholamines; at 25° 12 s.d. 18 × d min^{-1} (n = 2) radioactivity; 0·07 s.d. 0·04 μg (n = 3) catecholamines.

cholamines and total radioactivity. Chromaffin granules were isolated by the technique of Smith & Winkler (1967) and analysed for radioactive nucleotides using PEI-cellulose thin-layer chromatography (Stevens & others, 1972). Mean values of the percent increase above the prestimulation concentration for bovine and canine perfusions at 37° and 25° are summarized in Table 1. Only at 37° does the addition of carbachol result in a simultaneous increase in both radioactivity and catecholamine output. At 25° only catecholamine efflux was increased significantly above prestimulation concentrations.

Since the bovine gland is considerably larger than the canine gland the amount of catecholamines and radioactivity released per unit time into the effluent from the bovine gland are much greater. Therefore shorter intervals of time (30 s) were used in the measurement of radioactive and amine efflux from the bovine adrenal gland. Five min intervals were used for the canine adrenal gland.

An analysis of the radioactivity in chromaffin granules isolated from bovine and dog adrenal glands perfused at 37° revealed that considerable labelling of the granules had occurred at that temperature. Furthermore, a considerable amount of phosphorylation had occurred; ^{3}H-ATP represented 85–87% and 67 to 68% of the total phosphorylated radioactivity respectively in the bovine and dog granules. An analysis of the radioactivity in granules isolated from bovine or dog glands perfused at 25° failed to demonstrate measurable quantities of radioactivity in the granules. Consequently chromatographic analysis on these samples was not carried out. A temperature-dependent process for the conversion of adenosine to adenine nucleotides has been described previously in canine and human red blood cells (Van Belle, 1969). Although, the present studies do not provide information as to which step or steps in the labelling of the granular ATP are rate limiting, it would appear that one or more of these steps in adrenal chromaffin granules is a temperature-dependent enzymatic process.

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REFERENCES

Douglas, W. W. & Rubin, R. P. (1961). *J. Physiol.*, **159**, 40–57.
Kovacic, B. & Robinson, R. L. (1970). *J. Pharmac. exp. Ther.*, **175**, 178–182.
Robinson, R. L. (1966). *Ibid.*, **151**, 55–58.
Robinson, R. L. (1967). *Ibid.*, **156**, 252–257.
Rubin, R. P. & Jaanus, S. D. (1967). *Arch. exp. Path. Pharmak.*, **256**, 464–475.
Rubin, R. P. & Miele, E. (1968). *J. Pharmac. exp. Thér.*, **164**, 115–121.
Smith, A. D. & Winkler, H. (1967). *Biochem. J.*, **103**, 480–482.
Stevens, P., Robinson, R. L., Van Dyke, K. & Stitzel, R. E. (1972). *J. Pharmac. exp. Ther.*, **181**, 463–471.
Stevens, P., Robinson, R. L., Van Dyke, K. & Stitzel, R. E. (1975). *Pharmacology*, **13**, 40–55.
Trifaró, J. M., Poisner, A. M. & Douglas, W. W. (1967). *Biochem. Pharmac.*, **16**, 2095–2100.
Van Belle, H. (1969). *Biochim. biophys. Acta*, **192**, 124–132.