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Enhancement of the relative uptake of ^{18}F-FDG in mouse fibrosarcoma by rolipram

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The effect of rolipram, a selective phosphodiesterase type 4 inhibitor, on the uptake of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) in tumor tissue was examined in mice transplanted with NFSa fibrosarcoma. The uptake indexes of ^{18}F-FDG in the heart, skeletal muscle and brain remarkably decreased after treatment with 3 mg/kg of rolipram (heart: 13%, skeletal muscle: 14%, brain: 31%), but fibrosarcoma tissue showed only a 50% reduction in the uptake index of ^{18}F-FDG. The tumor/muscle ratio of radioactivity 30 min after ^{18}F-FDG injection was consequently enhanced from 1.9 to 6.5 by rolipram. This indicates the possible use of rolipram to enhance the sensitivity of tumor detection, as well as characterization of tumors in ^{18}F-FDG PET.

Key words: rolipram, fibrosarcoma, ^{18}F-fluorodeoxyglucose

INTRODUCTION

SEVERAL REPORTS have indicated stimulating effects of cyclic AMP on glucose metabolism in isolated tissues or cultured cells,[1–3] but we recently found suppressive effects of cAMP/PKA systems on glucose utilization in the intact rat brain[4] as well as mouse heart and skeletal muscle (in preparation). Microinjection of db cAMP, a cAMP analogue, into rat striatum decreased [^{14}C]-deoxyglucose uptake in this region. On the other hand, inhibition of PKA with Rp-cAMPs resulted in significant enhancement of [^{14}C]-DG uptake in the brain. Significant reduction of glucose utilization in intact heart and skeletal muscle was also observed in mice pretreated with rolipram, a phosphodiesterase type 4 inhibitor. As rolipram caused an increase in the intracellular cAMP concentration,[5] these results indicated an important role of cAMP/PKA systems in regulating glucose metabolism in the intact brain and peripheral tissues. ^{18}F-labeled fluorodeoxyglucose (^{18}F-FDG) has been commonly used for the diagnosis of various kinds of cancer.[6,7] It is of interest to know whether ^{18}F-FDG uptake in cancer is also regulated by the cAMP/PKA system. In this paper, we report relatively resistant ^{18}F-FGD uptake in mouse sarcoma against treatment with rolipram as compared with normal peripheral tissues.

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MATERIALS AND METHODS

Male C3H/HeMsNrsf mice (5 weeks old) were produced and maintained in specific pathogen-free (SPF) facilities in the National Institute of Radiological Sciences (Chiba, Japan). The tumor was a syngeneic NFSa fibrosarcoma, and generations 16 through 18 were transplanted intramuscularly into the right hind legs of mice 10 days before the tracer experiment.[8] The size of the tumor was about 8–10 mm in diameter in the tracer experiment. Rolipram was purchased from Tocris Cookson Ltd. (Bristol, UK) and suspended in saline containing 5% plant oil (HCO-60, Nikkol). ^{18}F-FDG was synthesized by the method previously reported, and dissolved in saline solution. Radiochemical purity was more than 99% and specific

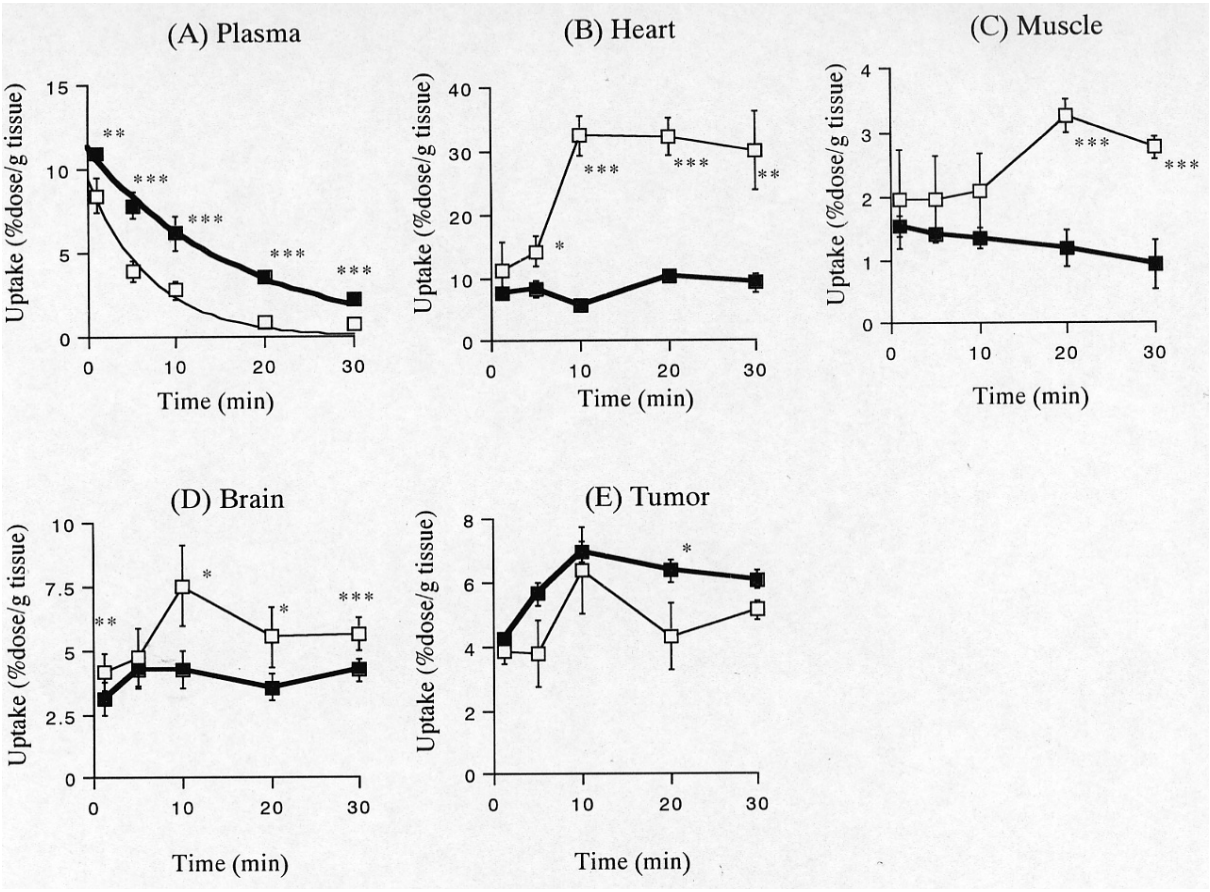

Fig. 1 The time course of radioactivity in plasma (A), heart (B), skeletal muscle (C), brain (D) and fibrosarcoma tumor (E) following injection of ^{18}F-FDG (⊟ control, ■ rolipram). An error bar express SD of each data point (n = 4). The significant difference between control and rolipram determined by t-test (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.005$). The line in plasma (A) expressed the most fitted single exponential function as $y = C_{p0} * \exp(-Kp * t)$ (Control: $C_{p0} = 9.44$, $Kp = 0.138$, Rolipram: $C_{p0} = 11.35$, $Kp = 0.059$).

Table 1 Effects of rolipram on the uptake index (A) and ratios of radioactivity in tumor by plasma or skeletal muscle (B) of ^{18}F-FDG in fibrosarcoma transplanted mice

(A) Uptake index

	Brain	Heart	Skeletal muscle	Tumor
Control	0.084 ± 0.004***	0.446 ± 0.091***	0.041 ± 0.003***	0.077 ± 0.010***
Rolipram	0.027 ± 0.002	0.057 ± 0.010	0.006 ± 0.002	0.038 ± 0.003
$\%\frac{Rolipram}{Control}$	31%	13%	14%	50%

(values are expressed average ± SD of 4 mice. ***: $p < 0.005$)

(B) Ratios of radioactivity in tumor by plasma or skeletal muscle

	Tumor/Plasma		Tumor/Skeletal muscle	
	Control	Rolipram	Control	Rolipram
1 min	0.466 ± 0.117	0.347 ± 0.094	1.693 ± 0.372	2.542 ± 0.854
5 min	0.957 ± 0.171	0.614 ± 0.269	1.978 ± 0.195	3.961 ± 0.236***
10 min	2.144 ± 0.267	1.130 ± 0.084**	2.536 ± 1.250	5.185 ± 0.306*
20 min	5.279 ± 1.536	1.798 ± 0.130*	1.335 ± 0.406	5.531 ± 0.948***
30 min	6.940 ± 0.852	2.397 ± 0.637***	1.867 ± 0.307	6.426 ± 2.382*

(values are expressed average ± SD of 4 mice. *: $p < 0.05$, **:$p < 0.01$, ***: $p < 0.005$)

radioactivity was more than 370 GBq/μmol.

Tumor bearing mice were intraperitonealy injected with rolipram (3 mg/kg) or saline 15 min prior to the tracer injection. The mice were intravenously injected with 0.2 m*l* of ^{18}F-FDG (10 MBq), and decapitated at 1, 5, 10, 20 and 30 min (n = 4 at each time point) after the tracer injection. The blood, brain, heart, tumor and muscle were sampled and weighed. The blood samples were centrifuged for 10 min and plasma samples were weighed. Radioactivity in each sample was measured by means of a well scintillation counter, and values were expressed as percent-injected dose per gram tissues with decay corrections.

According to the method previously reported,[9] indexes of relative glucose uptake in tissues (K) were estimated by the following function:

$$K = C*Kp/C_{p0}(1 - e^{-Kp})$$

Where C is the radioactivity concentration in the tissues 30 min after the tracer injection, C_{p0} is the initial radioactivity concentration in plasma and Kp is the plasma disappearance rate constant.

RESULTS

The time courses of the radioactivity concentration in plasma, brain, heart, skeletal muscle and tumor after injection of ^{18}F-FDG are shown in Figure 1. Rolipram significantly increased the plasma level of ^{18}F-FDG at any time after the tracer injection. In brain, heart and skeletal muscle, initial uptakes of ^{18}F-FDG slightly decreased after the administration of rolipram, and significantly lower accumulations of ^{18}F-FDG in these tissues were seen from 10 to 30 min after the tracer injection into rolipram treated mice. Rolipram decreased the uptake index of ^{18}F-FDG in brain, heart and skeletal muscle to 31, 13 and 14% of the control, respectively. In contrast, only about a 50% reduction was found in fibrosarcoma. On the other hand, rolipram slightly increased ^{18}F-FDG accumulations in fibrosarcoma. The relative ratios of the radioactivity concentration in tumor to those in normal tissues 30 min post injection of the tracer are shown in Table 1. Significant improvements in the tumor/heart and tumor/muscle ratios were seen after treatment with rolipram, although the tumor/plasma ratio was not improved.

DISCUSSION

In the present experiment, rolipram significantly decreased ^{18}F-FDG uptake in brain, heart and skeletal muscle, which was consistent with our previous observations in normal mice. As initial uptakes of ^{18}F-FDG in brain, heart and skeletal muscle were slightly decreased by rolipram, the process of transporting ^{18}F-FDG from plasma to these tissues including perfusion might be reduced, but the kinetics of ^{18}F-FDG in these tissues suggested that reduction in the phosphorylation process of ^{18}F-FDG by hexokinase (HK) seemed to be more pronounced. Since rolipram has been reported to increase intracellular cAMP content, stimulation of the PKA system might suppress HK activity in normal tissues.

^{18}F-FDG is by far the most commonly used PET agent for detection of malignant tumor as well as prognostic monitoring therapy. The uptake mechanism of ^{18}F-FDG in malignant cells is well known.[10] It has been reported that type II hexokinase is overexpressed in malignant cells regardless of whether the tissue of origin expresses this enzyme.[11,12] The current observation is that the effect of rolipram on ^{18}F-FDG uptake in tumor is weaker than that in normal tissues and may be partly due to higher hexokinase activity in the tumor. Studies on dose related changes in ^{18}F-FDG uptakes in tumor and normal tissues are needed.

Rolipram has been developed as an antidepressant drug, and a multicenter double-blind study of three different doses of rolipram in patients with major depressive disorders was completed.[13] There were no findings that might cast doubt on the safety of the dosages. Therefore, it might be possible to use rolipram as an enhancer to detect small malignant tumors in ^{18}F-FDG PET imaging, because it significantly decreased ^{18}F-FDG uptake in normal tissues such as heart and skeletal muscle.

In a solid tumor implant model, the activity of tumor hexokinase was reported to be a marker of tumor growth rate.[14] As a future study, it will be of interest to know whether the response of tumor uptake of ^{18}F-FDG caused by rolipram is related to cancer prognosis or malignancy.

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