The Effect of Pentoxifylline and Propentofylline(HWA-285) on Post-Ischemic Rat Brain

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Takano M., Ohtomo H., Izumiyama M., Kogure K., Takahashi T.*, Iwata R.* and Ido T.*
Department of Neurology, Institute of Brain Diseases, Tohoku University School of Medicine
Cyclotron and Radioisotope Center, Tohoku University*

Abstract

The experiment was performed to test the hypothesis whether if the treatment of xanthine derivatives, pentoxifylline and propentofylline can present the development of post-ischemic brain edema caused by four-vessel occlusion rat model on Pulsinelli-Brierley preparation.

An in vitro ^{31}P-NMR spectrometry, HPLC and multi-tracer autoradiography (ARG) by ^{18}F-fluoro-deoxyglucose, ^{14}C-iodoantipyrine or ^{14}C-propentofylline were applied to determine the changes in energy metabolism and CBF in rat brain tissues.

After 60 minutes of ischemia, blood supply to the brain was restored. If the animal was treated with saline, the brain fails to maintain CBF glucose uptake during the 60 minutes post-ischemic recirculation.

While, in animals treated with pentoxifylline (10,30mg/kg), propentofylline (30,50mg/kg) and three metabolites of propentofylline (A720287,A802751,A802831) (30,50mg/kg) intravenously at the time onset of reflow, cerebral metabolism included glucose uptake and CBF improved significantly and the effects showed dose-dependence by determination of cerebral adenine nucleotides. Pre-treatment with pentoxifylline (30mg/kg) or propentofylline (10mg/kg) showed same effects as after-treatment with the drugs.

ARG study showed that ^{14}C-propentofylline and/or its metabolites are passing a blood-brain barrier. Hemorheologic properties of the compounds including the improvement of RBC deformability might improve the microcirculation which could play a role in countract the brain edema.

Introduction

Five xanthine derivatives of pentoxifylline, propentofylline and three metabolites of propentofylline(A720287,A802751,A802831) are good inhibitors of cAMP and cGMP phosphodiesterases, and show the multiple effects on cerebral metabolism and blood flow. (Fig. 1 chemical structure) Pentoxifylline and propentofylline(HWA285) increase cerebral blood flow (CBF) by inhibiting the uptake of adenosine by rat cerebral capillaries[6], and improve of microcirculation induced by hemorhelogical effects such as improvement of RBC deformability. The compounds are known to prevent of the brain edema induced by bilateral carotid ligation in gerbils[2,3,4,5] or cold injury in the cat brain[1],

and normalize of mitochondrial function isolated from the ischemic rat brain bystimulation of cerebral metabolism.[7-12)]

Three metabolites of propentofylline, the hydroxy-metabolite A720287 and the acidic metabolites A802751 and A802831 show a series pharmaceutical properties including the increase of CBF, inhibition of platelet aggregation, lipid-lowering activity, improvement of RBC deformability and protection against ischemic brain damage etc.

We preformed the experiment to test the hypothesis whether if the treatment of xanthine derivatives can prevent the development of post-ischemic brain edema caused by four-vessel occlusion rat model.

Materials and Methods

Vertebral arteries of male Wistar rats, weighing between 250 to 300 g were electrorically coagulated on Pulsinelli-Brierleys preparation.[13)] Twenty-four hours preparation, the animals were anesthelized with ether, immolbilized with pancromium bromide(Mioblock®, Organon, 0.5mg/kg, i.p.). Following tracheotomy, the animals were mechanically ventilated with a Harvard Rodent respirator set at 56/min of the respiratory rate. Catheters were placed into the both femoral arteries for monitoring the systemic arterial blood pressure and for withdrawal of blood sample. Artificial $PaCO_2$ and pH were maintained 38 ± 4 Torr and 7.35± 0.05 by appropriate adjustment of tidal volume, and PaO_2 was kept 100 ± 10 Torr by adding oxygen to respiration air. Catheter was placed into the right femoral vein for heparinization by heparin Na(Novo,1000U/kg) to promote blood recirculation after ischemia and for injection of the drugs and isotopes. Body temperature was kept at 37°C using a thermo-controlled heating pads.

Total hemispheric ischemia was produced by temporary clips on both common carotid arteries, and electroencephologram(EEG) of the animal became isoelectric within 30 seconds after occlusion. After 60 minutes of ischemia, clips were removed and the blood supply to the brain was restored. The drug solution of 0.5ml was administered intravenously with 5 min either 5 min before ischemia, or immediately with the onset of reflow. Eighty four animals were divided into fourteen groups including the sham-operated control and the 0.5 ml of saline-treated group according to the sampling schedule shown in Fig. 2. At the end of post-ischemic recirculation, the brain sampling was done by Ponten's technique.[14)] The brain was frozen by pouring liquid nitrogen on the skull from which the scalp epicranium was peeled off and was taken out in a frozen state after 10 minutes when the distortion caused by the freezing technique disappeared.

Cortical tissues of 500 - 800 mg was used for the in vitro ^{31}P-NMR spectrometry, and 100 - 200 mg for the assays of ATP, ADP and AMP by high performance chromatography(HPLC).

1) ^{31}P-NMR spectrometry

^{31}P-NMR spectrography was applied in order to determine the changes in energy metabolism in ischemic rat brain tissue. The frozen cortical tissue was grind in a mortar filled with liquid nitrogen, and put into 10 mm NMR tube.

^{31}P-NMR spectra for adenosine triphosphate(ATP), phosphocreatine(PCr), sugar phosphates and inorganic phosphate(Pi) were accumulated on a Bruker CXP-300 FT-NMR spectrometer at 121.5 Mz, collecting 6,000 transients at -5°C. Then proton irradiation (2 watt) was applied for the increase of S/N ratio. An equipped transmitter provided 90-degree pulse widths of 30 μ second for the nucleus at a peak-to-peak voltage of 300V.[20] Chemical shift of Pi from the H_3PO_4 in cortical tissue was utilized as an index to the pH changes.

2) Assay of nucleotides by HPLC

The separation of nucleotides obtained from a rat brain perchloric acid (PCA) extract was performed by anion-exchange HPLC column (Hitachi gel #3013-N, 150mm × 4.0mm i.d.) at 70°C. The cortical tissue was homogenized in 3M PCA with use of a motor-driven glass homogenizer at -20°C. After added to chilled water, the homogenate was centrifuged at 12,000 rpm for 20 minutes at 0°C. The pH of the clear supernalant fluid was adjusted to neutral (pH 7) with ice-cold 2M $KHCO_3$. The $KClO_4$ precipitate was eliminated by centrifugation at 3,000 rpm for 15 minutes at 0°C, and the PCA extract was assayed.

The moble phrase consisted of 6%(v/v)CH_3CN in 0.06M NH_4Cl-0.01M KH_2PO_4-0.01M K_2HPO_4 for the assay of AMP, and 6%(v/v)CH_3CN in 0.18M NH_4Cl-0.027M KH_2PO_4 -0.027M K_2HPO_4 for the assays of ATP and ADP. The flow rate was set at 1ml/min, and the eluate was monitored by an absorbance detection at 260 nm. The compounds were identified by their retention times and quantified by peak area measurement by means of an on-line computing integrator.

3) Multi-labeled autoradiography

Effects of pentoxifylline (30mg/kg) and propentofylline (10mg/kg) were studied by means of multi-tracer autoradiography (ARG) using ^{18}F-fluorodeoxyglucose (FDG), ^{14}C-iodoantipyrine (IAP) and ^{14}C-propentofylline as indicators of glucose uptake, regional CBF and the cerebral distribution of propentofylline (Fig. 3 sampling schedule).

^{18}F-FDG (2 mCi) was administered intravenously 29 minutes before ^{14}C-IAP (10 μCi) or 30 minutes after ^{14}C-propentofylline (10 μCi). The animals were sacrificed and the brain was sliced at 30 μm in thickness. Less than 20 minutes after the decapitation, the slices were contacted to a X-ray film (Kodak NMC-1) for 8 hours to acquire images of glucose uptake. Forty-eight hours the decay out interval for ^{18}F, the second autoradiogram for CBF or the drug distribution were processed 14 days.[21]

Results

1) ^{31}P-NMR spectrometry and HPLC

Representatives of ^{31}P-NMR spectrogram are illustrated Fig. 4 and 5. As can be seen, saline treated animals are failed to maintain the CBF and cerebral energy metabolism after 60 minutes recirculation. The high energetic phosporic compounds (ATP,PCr) decreased rapidly and inorganic phosporus (Pi) increased significantly, so the ^{31}P-NMR spectra only showed the peak of Pi. In contrast, the animals treated with high dose of pentoxifylline(30mg/kg), propentofylline

(10mg/kg) and three metabolites of propentofylline A720287,A802751, A802831(50mg/kg) maintained CBF and energy state, so the spectra showed the high peaks of ATP and PCr.

By HPLC assay of cerebral adenine nucleotides, in animals treated with saline at the time onset of reflow, the values were as followed; ATP 0.099±0.005, ADP 0.154±0.028, AMP 0.802±0.082 (μmol/g, mean± SEM) and energy charge (E.C.) 0.115±0.0011 (mean±SEM).

In sham-operated control, the values were ATP 2.854±0.039, ADP 0.267±0.018, AMP 0.042±0.007 (μmol/g) and E.C. 0.943±0.003. In animals treated with pentoxifylline (30mg/kg), propentofylline (5,10mg/kg), A720287 (50mg/kg), A802751 (30,50mg/kg) and A802831 (30,50mg/kg) at the time onset of reflow, its ATP, AMP and E.C. values changed significantly different in comparison with the saline-treated control (Student's t-test, $p<0.05$). And in animals pretreated with pentoxyfylline (30mg/kg) and propentofylline (10mg/kg), the cerebral metabolism improved significantly different from the saline-control.

Dose-dependant effect was observed with pentoxifylline (10,30mg/kg) and A720287 (30,50mg/kg), in animals treated with highdose of the drug at the time onset of reflow, its values of ATP, AMP and E.C. were improved significantly different from the low-dose treated animals.

2) Multi-labeled autoradiography

Representatives of autoradiograms are illustrated in Fig. 6 and 7. If the animal was not treated with pentoxifylline (30mg/kg) or propentofylline (10mg/kg), the brain failed to maintain the post-ischemic blood flow and metabolism 60 minutes after ischemia remained far below normal. When the animals were treated by intravenous administration of either pentoxifylline or propentofylline, the post-ischemic recirculation and the metabolic activity of the brain appeared significantly high compaired to the non-treated group.

The regional CBF and glucose uptake of the drug-treated groups appeared some heterogenous distribution, while it colud be shown that ^{14}C-propentofylline and/or its metabolites were passing a blood-brain barrier and were distributed homogenously at the end of 60 minutes recirculation (Fig. 7). The regional CBF of the animal treated with the drug was increased in the cerebral cortex, hippocampus, thalamus and part of the basal ganglia. The dissociation of CBF and metabolism was observed in the early stages of ischemic rat brain.[16,22,23,24]

Discussion

Occlusion of all four major arteries supplying the rat brain decrease CBF sufficiently to produce ischemic insult on Pulsinelli-Brierley preparation. The advantages of this model are ease of preparation of large numbers of animals requisite for statistical analysis, and a high rate of ischemic neuronal damage which show isoelectric EEG within 30 seconds of ischmia. With this model, the cerebral metabolism in post-ischemic recirculation is possible to recover after

30 minutes of ischemia.[15] If the period of ischemia is prolonged to 60 minutes, it showed irreversible change following post-ischemic recirculation.[16,17]

Biochemically the disease stages of ischemia seem to progress in the following order:[15] 1) Stage of acidosis, 2) Stage of energy crisis, 3) Stage of disintegration, and 4) Stage of autolysis. The postischemic events from 2) Stage of energy crisis seem to correspond with the biochemically changes of 60 minutes recirculation after 60 minutes ischemia.

The disease stage of 2) energy crisis (depletion of ATP) gradually advances in the following order: 1) Phase of NAD^+ lacking in cytoplasma, 2) Phase of metabolic dissociation with mitochondria and cytoplasma, 3) Phase of injury on electron transport shuttle system in mitochondria. The consideration to change a therapeutical policy stepwise adapting at each stage and phrase is particularly important. Concerning Stage 2, the suitable therapy of Phrase 2 and 3 are the treatments with vasodilators, agents showed improvement of RBC deformability, reduction in blood viscosity and platelet-aggregation, anti-thrombotic agents, anti-coagulants, hyperosmotic agents, cerebral metabolism suppresing agents and anti-oxidants etc.[15,19]

In this experiment, pentoxifylline and propentofylline showed the prevention of the development of post-ischemic brain edema. The drugs are known to increase in CBF by inhibiting the adenosine uptake by rat cerebral capillaries. Hemorheologic properities of the drugs, such as improvement of RBC deformability, reduction of blood viscosity and platelet aggregation might improve the microcirculation which could play a role in countract the brain edema.

Modification of brain edema were demonstrated not only in this experiment, but also in studies of gerbils with bilaterally occluded carotids by treated with the drugs, but the mechanism of anti-edematous effect was not clear. In the gerbil-stroke model, both pentoxifylline and propentofylline were able to prolong its survival period significantly, and decrease water contents, changes of electrolytes (Na^+,K^+) and the mortalty. The post-ischemic reduction of Na^+-K^+-ATPase was prevented by administration with propentofylline and the effect on Na^+-K^+-ATPase might modificate of brain edema.

According to Cohen[3,8], one of mode of action of pentoxifylline in ameliorating ischemic brain may be increased metabolic capability secondly to mitochondrial hypertrophy in cerebral cortex and hippocampus. Five xanthine derivatives are good inhibitors of cAMP and cGMP phosphodiesterase, so the compounds show the mulitiple effects on cerebral metabolism and blood flow. Those effects might initiate an improvement in disturbed membrane permeability and in the function of brain cells by injury of ischemic insult.

Further investigation of the xanthine compounds on the effect of the edematous brain have to be performed to clear up the mechanism of anti-edematous effect of the compounds.

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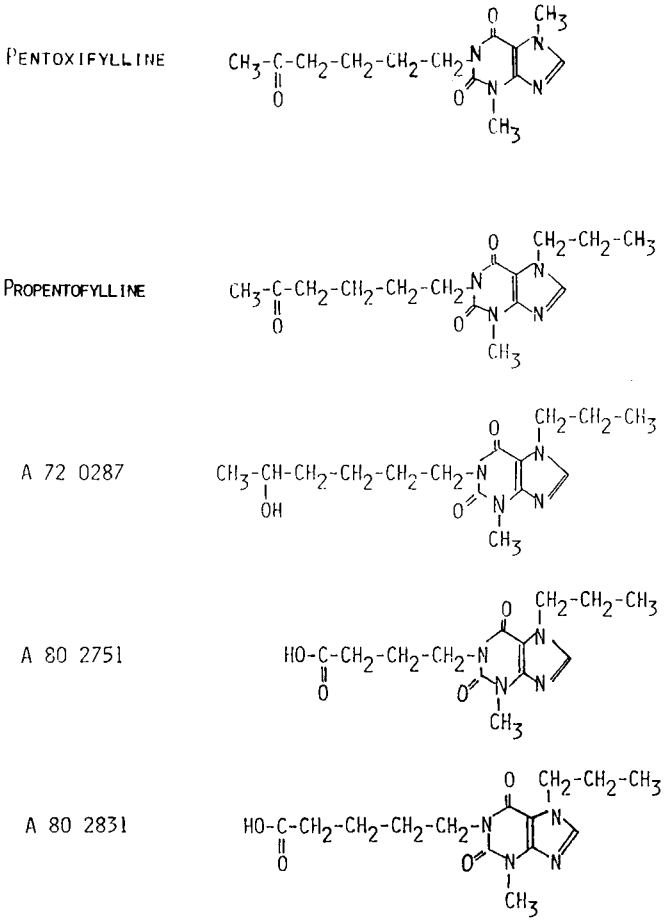

Fig. 1. Chemical structures of pentoxifylline, propentofylline and three metabolites of propentofylline.

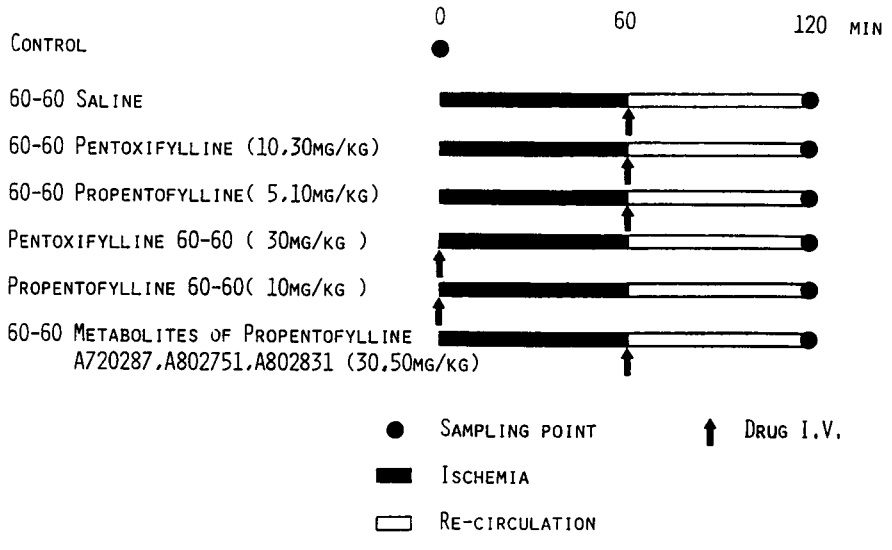

Fig. 2. Sampling schedule of ^{31}P-NMR and HPLC.

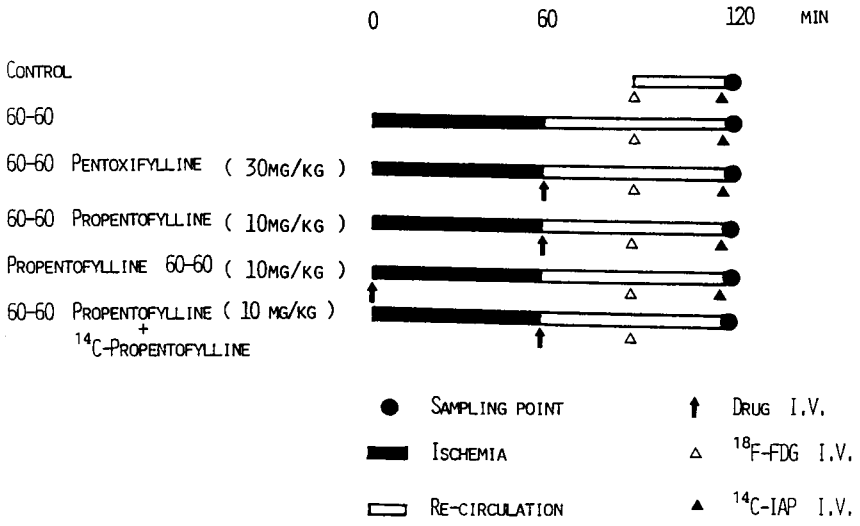

Fig. 3. Sampling schedule of double-tracer autoradiography.

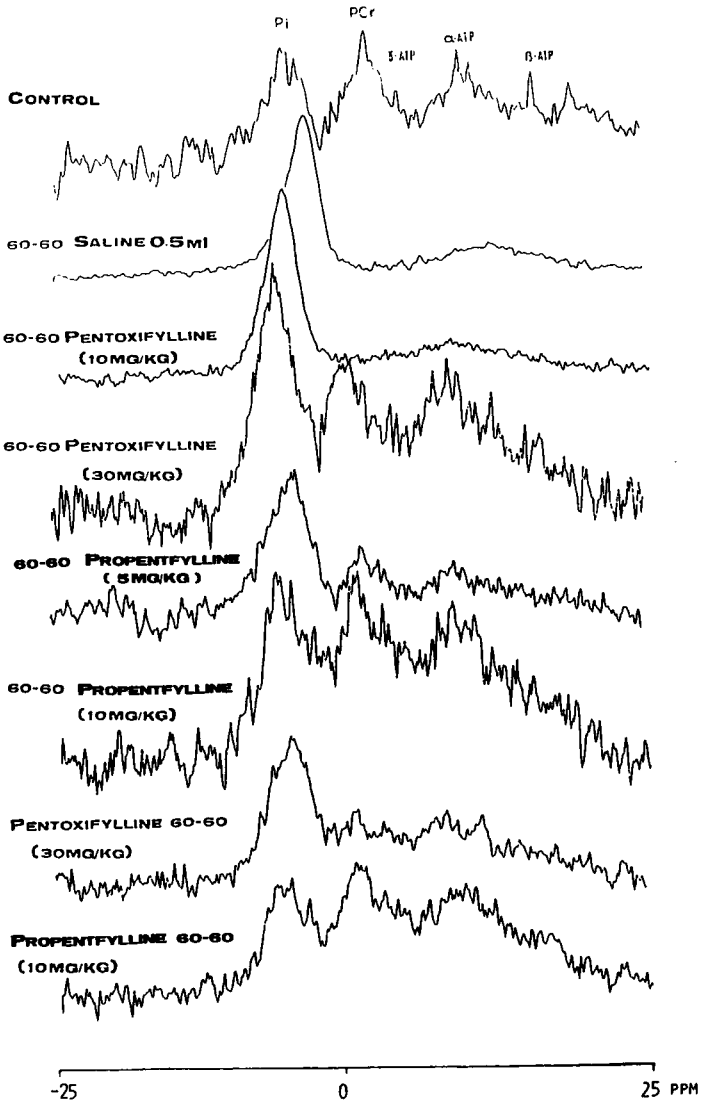

Fig. 4. Representatives of ^{31}P-NMR spectrograms of the rat brain treated

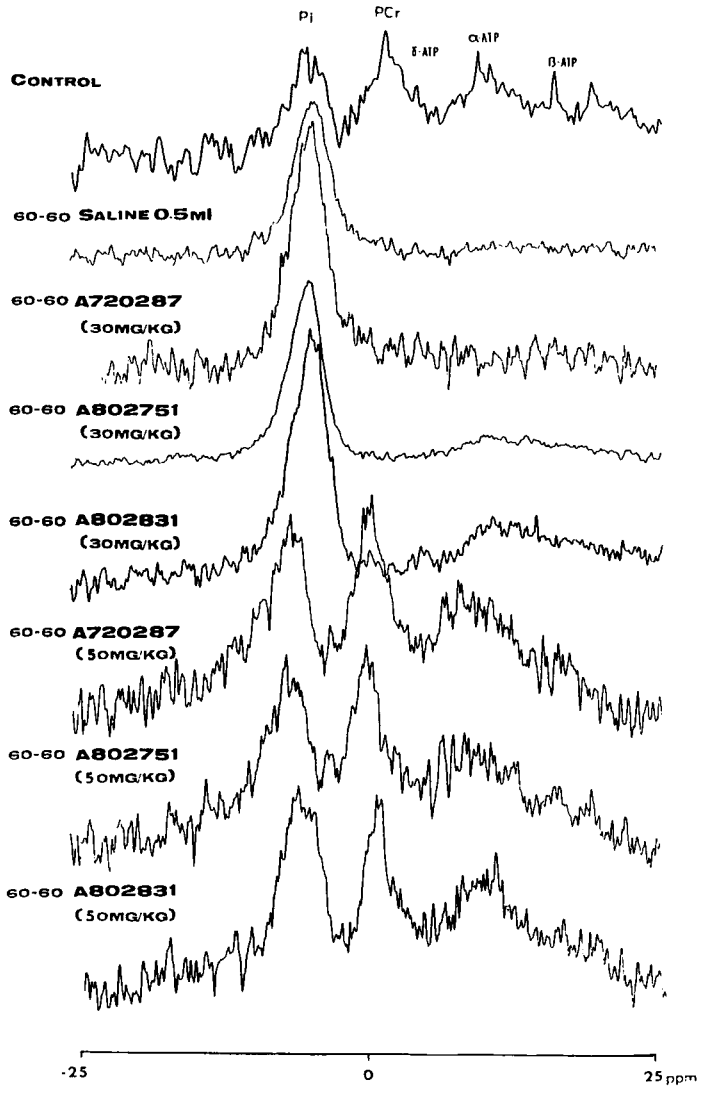

Fig. 5. Representatives of ^{31}P-NMR spectrograms of the rat brain treated with metabolites of propentofylline.

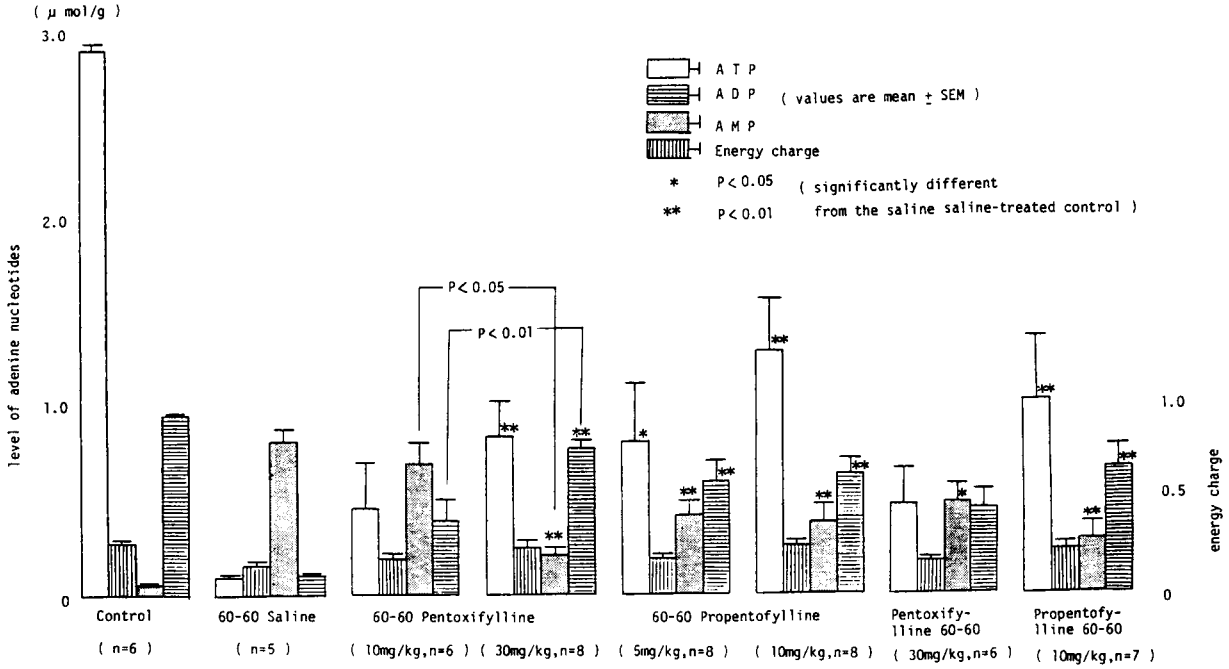

Fig. 6. The effect of pentoxifylline and propentofylline on rat cerebral adenine nucleotides and energy charge.

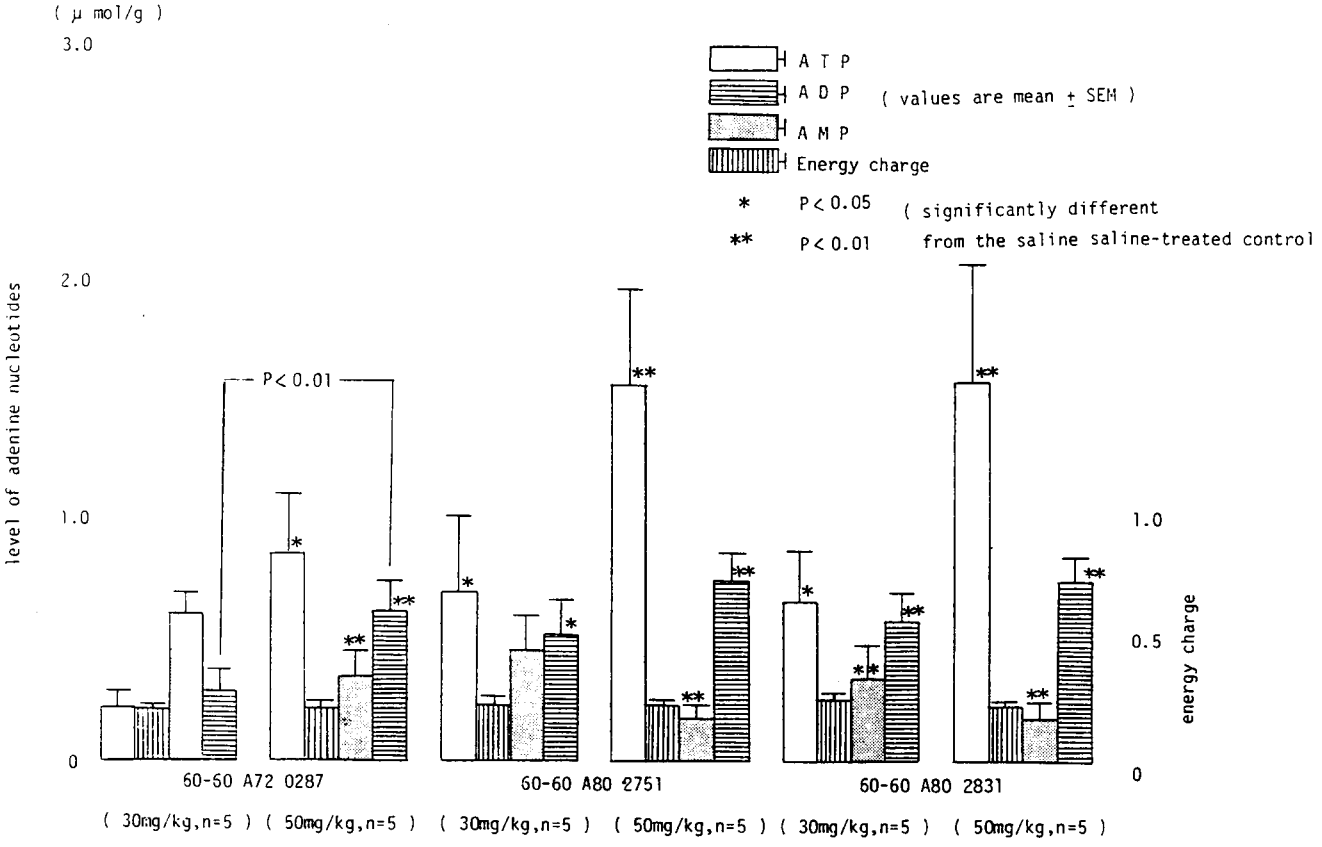

Fig. 7. The effect of three metabolites of propentofylline on rat cerebral adenine nucleotides and energy charge.

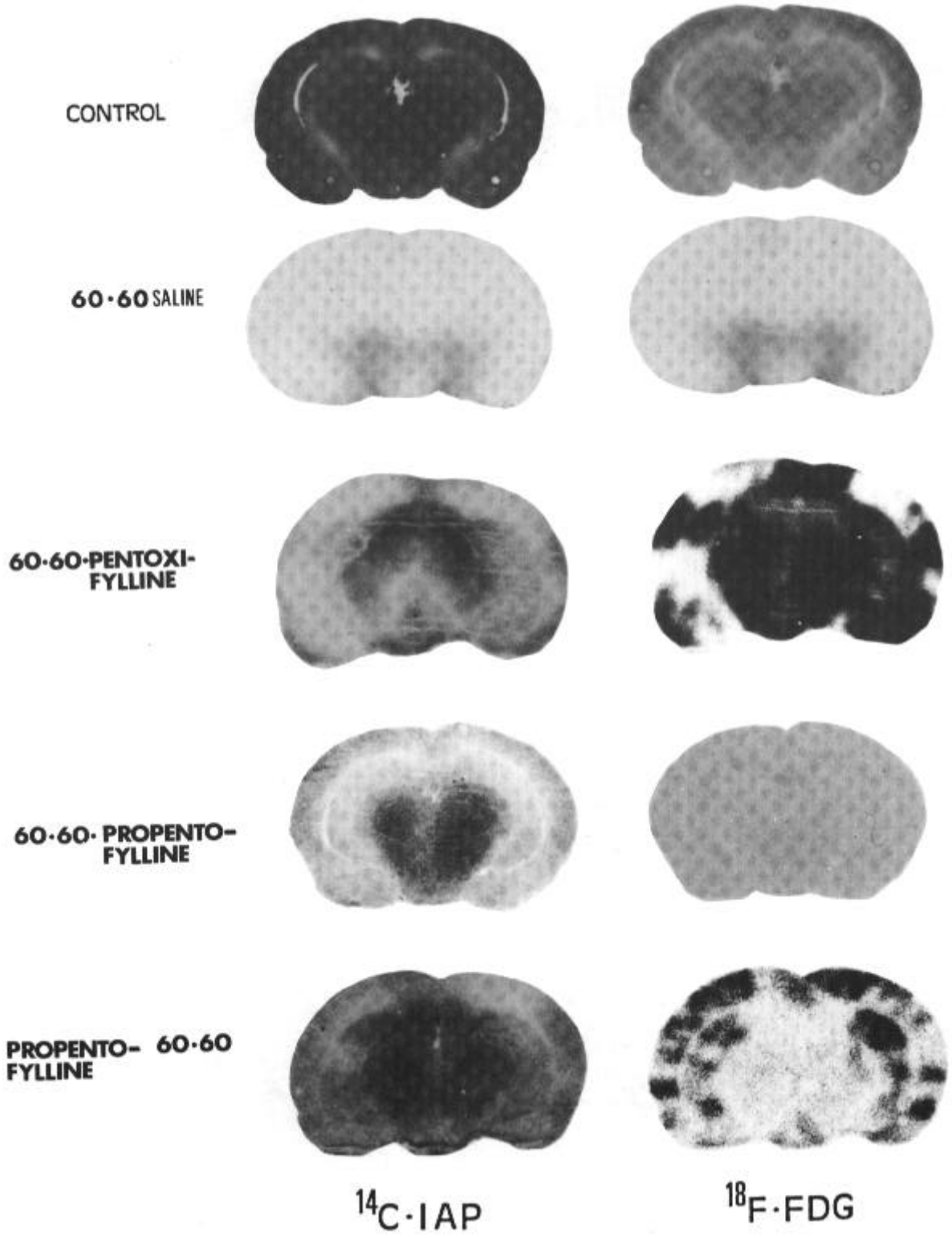

Fig. 8. Representatives of double-tracer autoradiograms.

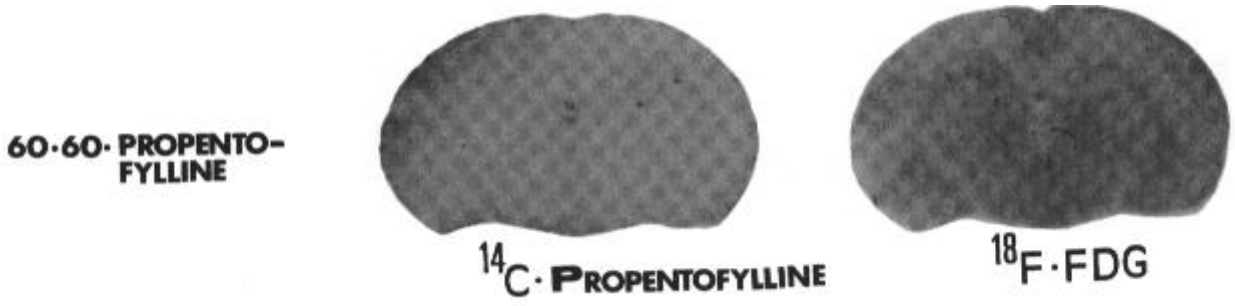

Fig. 9. Representative of double-tracer autoradiogram.