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Effects of In-vivo Administration of Taurine and HEPES on the Inflammatory Response in Rats

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Abstract—The effect of in-vivo administration of *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid (HEPES) and taurine on rat paw oedema and reactive oxidant production was examined. Carrageenan-induced paw oedema was attenuated following intraperitoneal injection of HEPES. Chemiluminescence production by isolated peripheral blood mononuclear cells (PBMC) was reduced in HEPES-treated rats. Taurine-treated rats did not exhibit attenuation of paw oedema using subcutaneous or intraperitoneal administration but intracerebroventricular administration produced a significant reduction at a dosage of 4·0 μmol. No reduction in chemiluminescence production was observed by PBMC using subcutaneous or intraperitoneal administration of taurine, but intracerebroventricular administration produced a significant reduction at a dosage of both 0·4 and 4·0 μmol. Intravenous injection of [^{14}C]HEPES or [^{3}H]taurine demonstrated rapid clearance with a significantly longer half-life of HEPES compared with taurine. These results support previous reports of anti-inflammatory activity of taurine when administered centrally. The lack of anti-inflammatory effect when taurine was administered subcutaneously or intraperitoneally may be a consequence of rapid distribution or clearance. The greater anti-inflammatory effects of HEPES compared with taurine may be due to its slower distribution or clearance in-vivo.

Taurine is one of the most abundant amino acids in the brain (Jacobsen & Smith 1968). The high levels of taurine in this organ have stimulated much research to establish possible functions for this compound. The role of taurine as a putative neurotransmitter was reviewed by Kuriyama (1979). McBride & Frederickson (1979) proposed taurine as a possible inhibitory transmitter in the cerebellum. The level of taurine is threefold higher than the level of γ-amino-butyric acid (GABA) in the molecular layer than the granular layer in the cerebellum of the rat.

The distribution of taurine injected systemically has been determined in several species. Machlin & Pearson (1957) injected [^{35}S]taurine into young chickens by the intramuscular route and reported the greatest recovery of the radiolabel to be in the heart, duodenum, spleen, gizzard, muscle and stomach while 16% was excreted. Awapara & Manz (1957) administered [^{35}S]taurine by intravenous injection into rats. Rapid uptake was exhibited by the kidney, spleen, liver and bone marrow whereas uptake by the heart and muscle was considerably slower. Long-term retention of taurine by the heart was indicated by the fact that seven days after initial injection there were still considerable quantities of [^{35}S]taurine present. Boquet & Fromageot (1965), studied the specific activity of taurine in various rat tissues following injection of tracer amounts of [^{35}S]taurine. Isotopic equilibrium was achieved between the plasma and liver within 30 min of administration. However, isotopic equilibrium between plasma, heart and muscle required five days. The rate of turnover of taurine was tissue-dependent, liver and kidney demonstrating a more rapid rate whereas that of the heart and muscle was markedly slower. The half-life ($t_{\frac{1}{2}}$) for taurine in the whole body of the rat was calculated as 12–13 days, corresponding to a daily renewal of 5·7% which was equivalent to 35 μmol taurine/100 g body weight. Following administration of exogenous taurine the rate of cellular uptake was not uniform but varied with cell type. Retention was also organ-dependent, with the tissue concentration which resulted from an equilibrium between biosynthesized and exogenous taurine replacing that which was lost from the cells or which was further metabolized (Jacobsen & Smith 1968).

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Bhattacharya & Sarkar (1986), reported intracerebroventricularly (i.c.v) administered taurine to be anti-inflammatory in-vivo. The basis on which this claim was made was the observation that there was significant attenuation of carrageenan-induced paw oedema in rats following i.c.v. administration of taurine. Taurine was administered at two dose levels (0·16 and 0·4 μmol); however, whereas attenuation of paw oedema was dose-dependent, the reduction was only significant at the 0·4 μmol level. No reduction in paw oedema was recorded following peripheral administration with similar dosages, and it was suggested that the effects of i.c.v. administered taurine was not a consequence of peripheral leakage. Since Bhattacharya & Sarkar (1986), did not demonstrate a peripheral effect of taurine administration, the aims of our work were: to confirm the previous anti-inflammatory effects of centrally administered taurine on rat paw oedema and to assess in-vivo effects of peripherally administered taurine or its structural analogue, HEPES, following intraperitoneal and subcutaneous (s.c.) administration; to measure reactive oxidant production by isolated peripheral blood mononuclear cells (PBMC) at the termination of the experimental time period as an index of the anti-inflammatory activity; to establish the rate of clearance of peripherally administered [^{3}H]taurine and [^{14}C]HEPES by the intravenous route and to relate the clearance of each of these compounds to observed in-vivo anti-inflammatory effects; and to measure the uptake of labelled taurine or HEPES into selected organs over a 90-min period.

Materials and Methods

Animals

Adult inbred Wistar rats of either sex, 120–180 g, obtained from the Bio.Resources Unit, Trinity College, Dublin 2, Ireland, were used in these studies. They were maintained on a standard laboratory chow diet and had free access to water.

Materials

[1,2-^{3}H]Taurine (17 Ci mmol^{-1}) and [^{14}C]HEPES (2·1 mCi mmol^{-1}) were obtained from the Radiochemicals Centre, Amersham, UK. Ecolite scintillation solution was supplied by ICN Biomedicals Inc., Radiochemicals Division, Irvine, CA, USA. Whatman No. 3 chromatography paper, 20 × 20 cm was purchased from Whatman Ltd, Maidstone, Kent, UK. Ethidium bromide, acridine orange, calcium ionophore A23187, carrageenan type IV (lambda), and Dowex-50W X 8 (200–400 mesh H^+ form), were purchased from Sigma Chemical Co., St Louis, MO, USA. Dulbecco's (10X) phosphate-buffered saline (PBS) was supplied by Gibco Ltd, Paisley, UK. Luminol was purchased from Lumac Medical Products Division, St Paul, MN, USA. Dextraven 150 injection in 0·9% saline was purchased from Fisons Ltd, Loughborough, UK. Pentobarbitone sodium (Sagatal) and midazolam were supplied by Boileu and Boyd (Dublin, Ireland). Sodium heparinized vacutainers were obtained from Becton-Dickinson & Co., Rutherford, NJ, USA. All other reagents were of analytical grade.

Carrageenan-induced paw oedema

Paw oedema was induced 30 min after injection of radiolabelled taurine or HEPES by injecting carrageenan (100 μL 1% suspension in 0·9% saline) below the plantar aponeurosis of the hind paw (Bhattacharya & Sarkar 1986). Paw volume was measured initially and 60 min following oedema inducement by displacement of water in a graduated cylinder.

Isolation of PBMC

Whole rat blood was collected into sodium heparinized vacutainers and diluted 1 : 2 (v/v) with dextraven 150. The samples were mixed by gentle inversion and the erythrocytes allowed to sediment at room temperature for 30–40 min. The cell-rich plasma layer was removed and diluted 1 : 2 (v/v) with 0.9% saline supplemented with 1 mM glucose. This suspension was centrifuged at 800 *g* for 5 min at 20°C in a Sorvall RT6000B centrifuge. The PBMC pelleted and residual erythrocytes in the cell fraction were lysed by sequential addition of 3 mL filtered deionized water and 1 mL 3·6% saline. The cells were further centrifuged at 800 *g* for 5 min at 20°C to remove erythrocyte ghosts. The supernatant was discarded and the PBMC resuspended in 0·9% saline containing 1 mM glucose.

Microscopic cell counting

Cell concentration and viability was assessed by viewing a suspension of cells in an ethidium bromide/acridine orange (EB/AO) solution on a Neubauer haemocytometer as previously described (McLoughlin et al 1991). Viability was shown to be approximately 99% and on the basis of cytology, greater than 98% of cells viewed were monocytes.

Chemiluminescence

Reactive oxidant production was measured using 100 μL 5×10^6 cells mL^{-1} by following chemiluminescence responses in a Lumac M2010 Biocounter. The cells were stimulated with 100 μL A23187 (2 μM) in the presence of 100 μL luminol (0·1 mM). Peak responses obtained after approximately 10 min at 37°C are reported (McLoughlin et al 1991).

Experimental

The animals were anaesthetized by intraperitoneal injection of pentobarbitone sodium (65 mg kg^{-1} body wt) or mixture of fluanisone/fentanyl citrate/midazolam (Flecknell 1987) and a femoral vein cannulated. Following anaesthetization and cannulation the rats were injected with 500 μL (0·4, 4·0, or 40 μmol, i.v., i.p. or s.c.) [^{3}H]taurine or (2·1, 21, or 210 μmol) [^{14}C]HEPES in 0·9% NaCl. For intracerebroventricular administration 10 μL taurine (0·4 or 4·0 μmol) was injected into the right lateral ventricle as previously described (Bhattacharya & Sarkar 1986). For intravenous administration of [^{3}H]taurine a second cannula was inserted into the contralateral femoral vein and the radiolabel administered via this cannula. For intravenous administration of [^{14}C]HEPES the route of administration was via the sub-lingual vein. Control rats were injected with 0·9% NaCl (500 μL). Following administration of [^{3}H]taurine or [^{14}C]HEPES, blood (250 μL) was drawn at regular intervals (5 or 10 min periods) up to 90 min. The level of radiolabel present in each sample was estimated by taking 200 μL and adjusting to 1 mL with distilled H_2O. Perchloric acid (100 μL 70% solution) was added, the sample vortexed (approx. 10 s) and refrigerated at 0°C for 10 min. They were vortexed again and centrifuged at 15 000 *g* for 2 min in a bench centrifuge (BHG Hermle Z 230 M). An aliquot of supernatant (800 μL) was counted in 4·2 mL Ecolite in a Packard 1500 Tricarb Scintillation Counter. Quenching by perchloric acid was estimated by counting [^{3}H]taurine or [^{14}C]HEPES standards with and without acid. All counts were related to μmol taurine or HEPES (mL blood)$^{-1}$. At the end of the experimental period the animals were killed while still anaesthetized by rapidly excising the heart. The liver, kidneys, spleen and brain were also removed. The level of radiolabel incorporated into each organ was estimated by homogenizing each organ in 10 vol 10% (w/v) ice-cold trichloroacetic acid. The homogenate was centrifuged at 8000 *g* for 30 min at 2–4°C in a Sorvall RC-5B refrigerated Superspeed centrifuge (Ida & Kuriyama 1983). An aliquot of supernatant (1 mL) was counted in 4 mL Ecolite and all counts related to total levels of ^{3}H or ^{14}C per organ as a percentage of the original injection. Following intracerebroventricular administration of [^{3}H]taurine, the radiolabel incorporated into each organ studied was identified by ion-exchange (Ida & Kuriyama 1983) and paper chromatography in a butan-1-ol : acetic acid : water (12 : 3 : 5 v/v) system (Ersser & Smith 1976) following extraction and lyophylization. For intraperitoneal and subcutaneous administration, ^{3}H and ^{14}C were identified by paper chromatography and liquid scintillation counting.

In the experiments where only chemiluminescence was measured, the animals were not cannulated and therefore blood samples were not taken to assess the levels of ^{3}H or

Table 1. Effect of intraperitoneally administered HEPES on carrageenan-induced paw oedema and chemiluminescence production by PBMC.

	Dose		
	2·1 μmol	21 μmol	210 μmol
Carrageenan-induced paw oedema			
Control	46·1 ± 2·63 (8)	39·5 ± 1·97 (16)	36·8 ± 1·64 (8)
Experimental	32·2 ± 2·63 (8)*	25·0 ± 0·66 (16)***	17·8 ± 3·95 (8)**
Chemiluminescence production			
Control	100 ± 5·83 (5)	100 ± 2·54 (9)	100 ± 26·1 (2)
Experimental	54·0 ± 8·27 (5)*	54·4 ± 4·35 (9)**	26·1 ± 9·41 (3)*

Results expressed as mean ± s.e.m. of three determinations in each case. Number of animals shown in parentheses. The mean chemiluminescence response for each set of controls were taken as 100% and all experimental readings were expressed as % control. $^*P < 0{\cdot}05$,$^{**}P < 0{\cdot}005$,$^{***}P < 0{\cdot}001$ compared with controls.

^{14}C. In those cases, the anaesthetized animals were exsanguinated by cardiac puncture at the end of the experimental period to collect PBMC as described above.

Statistical analysis

The results in Tables 1 and 2 show the mean ± s.e.m with number of experiments identified in each case. Statistical analysis was by Student's *t*-test for paired data, *P* values of $< 0{\cdot}05$ were considered significant. For unpaired data analysis, statistical significance was assessed by the Mann-Whitney U test and *P* values of $< 0{\cdot}05$ were considered significant. The pharmacokinetic parameters of half-life ($t_{\frac{1}{2}}$) and elimination rate constant (k_e) for taurine and HEPES following intravenous administration of [^{3}H]taurine or [^{14}C]HEPES were assessed by the program JANA (Dunne 1985). The AUC_{0-50} was computed using the linear trapezoidal rule. The $AUC_{0-\infty}$ was estimated by exponential extrapolation.

Results and Discussion

There are a number of papers suggesting that zwitterionic buffers can cause a reduction in observed reactive oxidants when the respiratory burst is stimulated by different methods. Schroeder et al (1978) observed that Tris and Bicine caused a reduction in chemiluminescence production by the H_2O_2-microperoxidase and H_2O_2-haematin systems. No explanation was offered for this observation. Harrison et al (1978) demonstrated a reduction in chemiluminescence production by a myeloperoxidase-peroxide-chloride system in the presence of 50 mM taurine. They proposed that this was due to trapping of HOCl by taurine, which prevented the production of singlet oxygen. Brestel (1985) reported that taurine inhibited luminol-amplified chemiluminescence production over a concentration range of 2·5 μM–2·5 mM and Kwon et al (1987) showed a concentration-dependent decrease in opsonized zymosan-stimulated chemiluminescence up to 50 mM HEPES. McLoughlin et al (1991) showed a reduction of A23187-stimulated chemiluminescence in neutrophils in the presence of 10 mM HEPES or 40 mM taurine. There was also a concomitant increase in myeloperoxidase activity. It was suggested that the reduction in chemiluminescence may be due to the sequestering of HOCl to form chloramines, thereby lifting possible end-product inhibition on myeloperoxidase as previously proposed by Naskalski (1977) in his studies on the effect of taurine on myeloperoxidase activity. We also showed that a number of zwitterionic compounds including HEPES and taurine at a concentration of 10 mM could result in reduced neutrophil chemiluminescence and enhanced myeloperoxidase activity (Stapleton & Bloomfield 1993). It was proposed that formation of taurine- or HEPES-chloramine may prevent the production of other reactive oxidant species which can contribute to chemiluminescence observed on stimulation of neutrophils. In addition to reduced chemiluminescence production observed in A23187-stimulated neutrophils in the presence of HEPES or taurine, we showed reduced leukotriene B_4 levels in the supernatants of stimu-

Table 2. Effect of taurine on carrageenan-induced paw oedema and chemiluminescence production by PBMC.

	Dose (s.c.)		Dose (i.p.)
	4·0 μmol	40 μmol	40 μmol
Carrageenan-induced paw oedema			
Control	33·0 ± 1·63 (6)	38·0 ± 0·63 (10)	
Experimental	27·0 ± 2·80 (6)	35·0 ± 0·79 (10)	
Chemiluminescence production			
Control	100 ± 7·01 (6)		100 ± 9·60 (5)
Experimental	101 ± 8·90 (6)		100·86 ± 5·76 (5)

Results expressed as mean ± s.e.m. of three determinations in each case. Number of animals shown in parentheses.

lated neutrophils (McLoughlin et al 1991; Stapleton & Bloomfield 1993). This was believed to be due to the increased myeloperoxidase activity which was previously shown by Henderson et al (1982) to cause catabolism of leukotriene B_4 in horse eosinophils. In our studies, we did not believe that the effect of taurine or HEPES was due to impairment of neutrophil function, since we showed no effect of these compounds on degranulation (McLoughlin et al 1991). In this study, the intraperitoneal administration of HEPES (2·1–210 μmol) resulted in a significant reduction in carrageenan-induced paw oedema in rats (Table 1). This attenuation in paw oedema was shown to be concentration-dependent. Chemiluminescence was also significantly decreased following intraperitoneal (2·1–210 μmol) administration. However, a dose-related reduction in reactive oxidant production was not observed between 2·1 and 21 μmol HEPES (Table 1). A significant reduction in chemiluminescence production was also observed following administration of 21 μmol HEPES by the subcutaneous route ($100 \pm 5{\cdot}88$ vs $56{\cdot}86 \pm 11{\cdot}76\%$, $P < 0{\cdot}05$). Both these effects are anti-inflammatory and therefore these results indicate that HEPES possesses anti-inflammatory activity in-vivo.

Centrally administered taurine (0·4 and 4·0 μmol) reduced paw oedema in a concentration-dependent manner. However, while previous studies have shown a significant reduction at the 0·4 μmol level (Bhattacharya & Sarkar 1986), this study showed significance only at 4·0 μmol ($46{\cdot}05 \pm 3{\cdot}95$ vs $21{\cdot}05 \pm 3{\cdot}95\%$, $P < 0{\cdot}05$). We also showed that chemiluminescence production demonstrated a significant reduction (100 ± 2.53 vs $40.51 \pm 2{\cdot}53\%$ and $100 \pm 8{\cdot}86$ vs $46{\cdot}84 \pm 21{\cdot}52\%$, $P < 0{\cdot}05$) following centrally administered taurine at both the 0·4 and 4·0 μmol doses, respectively. When taurine was administered subcutaneously (4·0 μmol) and intraperitoneally (40 μmol) small attenuations in carrageenan-induced paw oedema were observed which were non-significant (Table 2). Chemiluminescence production was not reduced following subcutaneous (4·0 μmol) or intraperitoneal (4·0 μmol) administration of taurine.

To establish the relationship between the effects on carrageenan-induced paw oedema and reactive oxidant production, the rate of clearance of [^{3}H]taurine or [^{14}C]HEPES following intravenous administration was assessed. The rate of clearance of 40 μmol [^{3}H]taurine or 21 μmol [^{14}C]HEPES following intravenous administration was shown to follow first-order kinetics. The initial sample taken was at 5 min and the concentrations of [^{3}H]taurine and [^{14}C]HEPES in the blood were found to be 0·460 and 0·256 μmol mL^{-1}, respectively. The mean blood volume of the rats used in these studies was approximately 7 mL, therefore the approximate total amount of each compound in the blood 5 min following injection was 3·220 μmol taurine and 1·792 μmol HEPES. Comparing these amounts with the original administered 40 μmol taurine or 21 μmol HEPES would suggest that 94·09 and 93·80%, respectively, of the exogenous taurine or HEPES was cleared or distributed in the tissues in the initial 5 min after administration. Awapara & Manz (1957), reported intravenous taurine to be rapidly taken up by kidney, spleen, liver and bone marrow in rats. Neither the tissue levels nor the amounts excreted were measured following intravenous administration of either [^{3}H]taurine or [^{14}C]HEPES in this study. Pharmacokinetic analysis by JANA showed the $t_{\frac{1}{2}}$ value for HEPES to be greater than that of taurine (Table 3). The k_e value calculated by JANA was 0·0492 μmol min^{-1} for taurine compared with 0·0168 μmol min^{-1} for HEPES. This indicated a significantly slower clearance for the synthetic analogue in comparison with the natural compound.

Table 3. Clearance of [^{14}C]HEPES and [^{3}H]taurine following intravenous injection.

Parameters	[^{14}C]HEPES (21 μmol)	[^{3}H]Taurine (40 μmol)
k_e (μmol min^{-1})	0·0168	0·0492*
AUC_{0-50} (μmol mL^{-1} min)	6·84	7·67
$AUC_{0-\infty}$ (μmol mL^{-1} min)	14·74	9·57
$t_{\frac{1}{2}}$ (min)	44·48	18·08

*$P < 0{\cdot}001$ compared with corresponding value for the [^{14}C]HEPES experiment.

Tissue levels of [^{3}H]taurine following administration of 40 μmol by the intraperitoneal or subcutaneous route (Table 4) indicate tissue uptake of exogenous taurine to be negligible for brain, heart and spleen over a 90-min period. For each route of administration, the percentage of label recovered in these organs, as identified by paper chromatography and liquid scintillation counting, was less than 0·5% of the original injection in each case. The percentage recovery was slightly higher in kidney when the route of administration was subcutaneous rather than intraperitoneal. The highest recovery was recorded in the liver with a marked difference in [^{3}H]taurine recovered ($20{\cdot}25 \pm 0{\cdot}51\%$) following intraperitoneal administration compared with the subcutaneous route ($7{\cdot}63 \pm 0{\cdot}31\%$). Taurine distribution following intracerebroventricular administration of [^{3}H]taurine (0·4 μmol) showed the label to predominately remain in the brain (Table 4).

HEPES was identified 90 min following intraperitoneal or subcutaneous administration of 21 μmol [^{14}C]HEPES only in the kidneys, but was further metabolized in the liver (Table 5). However, the techniques used in this study were unable to identify the ^{14}C-labelled compound detected in brain, heart and spleen due to low initial counts and a high percentage quench.

The results of this study represent preliminary findings on the effect of in-vivo administration of HEPES and taurine on the inflammatory response. This study confirms an anti-inflammatory effect of taurine when administered centrally. It supports a previous study which indicated an anti-inflammatory effect of intracerebroventricular administered taurine (Bhattacharya & Sarkar 1986). However, the dose at which attenuation of paw oedema was achieved in this study was 10 times that previously reported. The weight range for the rats was similar in both studies (120–180 g). However, our studies differed in experimental conditions. Bhattacharya & Sarkar (1986) cannulated the rats under anaesthetic following administration of pentobarbitone sodium and allowed the animals to recover for one week before administering taurine to the conscious animals. We

Table 4. Distribution (%) of [^{3}H]taurine 90 min after intraperitoneal, subcutaneous or intracerebroventricular administration.

Tissue	Intraperitoneal (40 μmol)	Subcutaneous (40 μmol)	Intracerebroventricular (0·4 μmol)
Brain	0·04 ± 0·01 (4)	0·04 ± 0·01 (3)	78·83 ± 12·44 (4)
Heart	0·20 ± 0·03 (4)	0·16 ± 0·01 (3)	0·52 ± 0·07 (4)
Spleen	0·26 ± 0·02 (2)	0·48 ± 0·11 (2)	Not analysed
Kidney	1·78 ± 0·06 (3)	2·69 ± 1·22 (3)	3·93 ± 1·87 (4)
Liver	20·25 ± 0·51 (3)	7·36 ± 0·31 (3)	6·84 ± 2·49 (4)

Results expressed as mean ± s.e.m. of three determinations in each case. Number of animals shown in parentheses. Taurine was identified in each tissue by paper chromatography.

administered taurine while the animals were anaesthetized following pentobarbitone sodium administraion. Reduced chemiluminescence production by isolated PBMC following central administration of taurine also substantiates the in-vivo anti-inflammatory effect of centrally administered taurine.

It is not clearly understood how centrally administered taurine exerts its observed anti-inflammatory effects. It was proposed by Bhattacharya & Sarkar (1986) that intracerebroventricularly administered taurine may act as an anti-inflammatory agent through association with central 5-HT, noradrenaline or histamine which all have been shown to exert a modulatory inhibitory influence over peripheral inflammation. While they showed no effect with peripherally administered taurine, it was demonstrated in the present study that when the route of administration was subcutaneous (4·0 μmol) or intraperitoneal (40 μmol), taurine did not exhibit anti-inflammatory properties in-vivo. This may be due to the rapid clearance of taurine from the system resulting in low mean blood levels. Identification of ^{3}H by paper chromatography and liquid scintillation counting following either intraperitoneal or subcutaneous administration of [^{3}H]taurine indicated no metabolism of injected taurine in the organs examined (data not shown). [^{14}C]HEPES was identified in the kidneys but appeared to be further metabolized in the liver, as a ^{14}C-rich compound with an R_f value greater than that for HEPES was demonstrated using paper chromatography and liquid scintillation counting (data not shown). The metabolite was not further identified. The uptake of [^{3}H]taurine into the liver was 20·25% using the intraperitoneal route of administration, while the level of ^{14}C detected following intraperitoneal administration of [^{14}C]HEPES was only 2·41% (Tables 4, 5). This would suggest that taurine is more efficiently distributed and may account for the lack of anti-inflammatory activity of in-vivo administered taurine by the peripheral route compared with HEPES. The anti-inflammatory effects demonstrated by HEPES in this study following both subcutaneous and intraperitoneal administration may be a consequence of its slower clearance in-vivo. Therefore it is proposed that while taurine and HEPES are anti-inflammatory in-vitro (McLoughlin et al 1991), taurine possesses anti-inflammatory activity in-vivo only when administered by the intracerebroventricular route, although HEPES is anti-inflammatory when administered peripherally. In addition, we have also demonstrated that zwitterions other than HEPES can modulate the inflammatory response in-vitro (Stapleton & Bloomfield 1993). Finally, we believe that the differences observed on the effects in this study with exogenous HEPES and taurine may be a result of the more rapid tissue distribution or clearance of the natural product and prolonged plasma half-life of the synthetic analogue HEPES in-vivo. There were no apparent adverse effects following HEPES administration in rats. In fact, the LD50 by intravenous and oral routes were shown to be greater than 3 and greater than 5 g kg^{-1} respectively (F. J. Bloomfield, personal communication). These facts, coupled with the slow clearance of blood HEPES, may suggest a new therapeutic approach to inflammatory diseases.

Table 5. Tissue distribution (%) of ^{14}C in rats 90 min after intraperitoneal or subcutaneous administration of 21 μmol [^{14}C]HEPES.

Organ	Intraperitoneal	Subcutaneous
Brain	0·23 ± 0·24 (4)	0·15 ± 0·21 (3)
Heart	0·13 ± 0·13 (4)	0·21 ± 0·21 (2)
Spleen	0·28 ± 0·22 (3)	0·04 ± 0·06 (3)
Kidney*	0·44 ± 0·19 (2)	2·44 ± 0·26 (2)
Liver	2·41 ± 1·21 (3)	4·78 ± 3·49 (4)

Results expressed as mean ± s.e.m. of three determinations in each case. Number of animals in parentheses. *[^{14}C]HEPES was identified by paper chromatography.

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