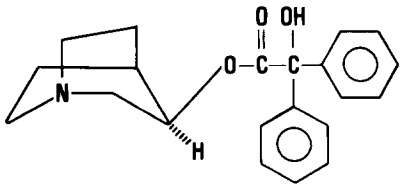
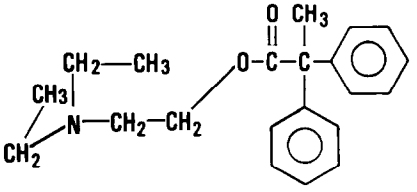


QNB



APROPHEN

Fig. 4. Chemical structures of QNB and aprophen.

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The effect of lithium on rat erythrocyte choline, glycine and glutathione levels

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The concentration of red blood cell (RBC) choline increases in patients as a result of lithium treatment [1, 2]. This accumulation of choline reaches steady-state levels 8 to 10-fold above baseline after 4–6 weeks of lithium treatment [1, 3, 4]. Lithium enhances brain acetylcholine (ACh) levels in rats following a pulsed dose of [$^2H_4$]choline [5]. Since choline is a precursor of ACh, and both RBC and neuronal membranes share many similarities, changes in RBC choline may parallel increased neuronal availability of ACh during lithium treatment.

Lithium has also been shown to increase the concentration of glycine in the RBC of manic-depressive patients [3, 6, 7] and the RBC and brain of rats [8]. Glycine is an inhibitory neurotransmitter and is also a precursor of RBC glutathione (GSH). If lithium increases RBC glycine, then these changes may affect RBC GSH levels. GSH is important for cell survival because it maintains the reducing potential within erythrocytes.

Free choline, glycine and GSH levels were measured in rat erythrocytes after lithium treatment with a view to develop an animal model in which to extend previous findings of lithium-induced changes in human erythrocytes. RBC metabolite concentrations were measured using proton magnetic resonance (^{1}H NMR) spectroscopy in conjunction with the spin-echo pulse sequence.

Materials and methods

Fourteen adult male Sprague–Dawley rats (390–480 g)

Table 1. Red cell choline, glycine and glutathione levels in saline and lithium-treated rats*

Treatment	Red cell concentration mM packed cells			
	Lithium	Choline	Glycine	Glutathione
Saline (n = 7)	—	0.0338 ± 0.0081	0.564 ± 0.149	1.01 ± 0.08
Lithium (n = 7)	0.547 ± 0.025	0.0721 ± 0.0076†	0.557 ± 0.101	1.08 ± 0.08

* Lithium (0.94 mmole/kg per day) was injected s.c. for 3 weeks. All rats were sacrificed 4 hr after the last injection. Each value is a mean ± S.E.

† $P < 0.005$ (analysis of variance, $F(1,12) = 11.83$).

were given daily s.c. injections of lithium chloride (Ajax Chemical) or isotonic saline for 21 days. Seven rats received saline (0.4 ml/kg, 0.154 M NaCl) and the other seven received 0.94 mmole/kg lithium per day (0.4 ml/kg, 100 mg/ml LiCl). The rats were housed individually in a temperature-controlled animal colony (20° ± 2°) with a 12 hr day–night cycle and were maintained on *ad lib.* access to food cubes (Allied Lab) and tap water.

The rats were sacrificed by decapitation 4 hr after their last injection on treatment day 21. Whole blood (10 ml) was collected into two heparinized tubes. Three ml of blood was collected into one tube for plasma lithium determination and the rest was collected into another tube containing 3 ml of ice-cold isotonic saline. Both tubes were immediately centrifuged (1000 *g* for 6 min) and the undiluted plasma from the first tube was decanted and stored frozen. The supernatant and buffy coat were aspirated and discarded from the second tube and the erythrocytes were again washed in saline. The supernatant was discarded and the remaining RBC pellet (2 ml, hematocrit 0.85) was decanted into encoded containers and stored for approximately 2 hr at 4° until assayed.

Fresh, unlysed erythrocytes were prepared for ^{1}H NMR as previously described for human lysed cells [9]. Spectra were recorded at 400 MHz using a Bruker WM400 spectrometer operated in the pulsed Fourier transform mode in conjunction with the spin–echo pulse sequence. A capillary containing tetramethylsilane (TMS) in deuterochloroform was placed within the sample tube and served both as chemical shift reference and concentration standard.

The *N*-methyl, glycyl-methylene and β-glutamyl methylene resonance intensities of choline, glycine and glutathione, respectively, were divided by the resonance intensity of TMS to give integral ratios. Integral ratios were then divided by the slope of the appropriate standard curve to give the concentrations (choline: 0.43; glycine: 3.16 and glutathione: 3.47).

RBC lithium concentrations were measured in the supernatant from pelleted cells (0.3 ml) diluted to 5 ml with 5% w/v trichloroacetic acid. Lithium standards were similarly prepared in a matrix of Bank blood. RBC and plasma lithium concentrations were determined using an atomic absorption spectrophotometer (Varian Techtron Model AA6) operated in the emission mode. RBC concentrations are expressed in mmole/l. of packed cells.

After the completion of all determinations the code was broken and the data were analysed using analysis of variance (ANOVA) and least-squares linear regression analysis.

Results and discussion

The mean RBC concentrations of choline, glycine and glutathione (GSH) from saline and lithium-treated rats are shown in Table 1. After 3 weeks of lithium treatment there was a 114% increase in RBC choline levels (0.072 mmole/l.) compared to the saline controls (0.034 mmole/l., $P < 0.005$). Although this dosage of lithium produced marked polyuria, the mean body weights of the lithium-treated rats were similar to controls at the beginning (420 vs 422 g) and after 21 days of treatment (455 vs 457 g). Several investigators have reported increased RBC choline levels in patients maintained on lithium [1–4]. To our knowledge this is the first study to report increased choline levels in rat erythrocytes as a result of lithium treatment. These data are in contrast to those reported by Wurtman and co-workers [10] and Jope *et al.* [11] in which choline levels were not elevated in rats maintained on a diet containing lithium (50–60 mmole of lithium per kg of food). The different findings are most likely due to procedural (dosage and route of lithium administration) rather than analytical differences [12].

The mean plasma lithium level was 0.77 mM and the RBC lithium level was 0.55 mM. Paradoxically, the increase in RBC choline was inversely related with plasma lithium ($r = -0.58$). A negative correlation has also been reported in patients [2, 3] and may indicate that the lithium-induced choline increase involves at least two molecular mechanisms [13]. The mechanisms involved in the accumulation of choline are not fully understood, but it has been shown that lithium inhibits both RBC choline influx and efflux [14, 15].

No differences were detected in the RBC glycine or GSH concentrations between saline and lithium-treated rats (Table 1). Since the inter-relationships between glycine and GSH levels were similar in both saline ($r = 0.58$) and lithium-treated rats ($r = 0.59$), these results also indicate that lithium did not affect the concentration of either metabolite. These data, therefore, do not support a previous finding of elevated RBC glycine levels in rats (17%) treated with lithium [8]. Although RBC glycine levels have been reported to be higher in lithium-treated patients [3, 6, 7], preliminary results from our laboratory have not been able to confirm this finding (in preparation).

In summary, no differences were detected in red cell glycine or glutathione levels between saline and lithium-treated rats. However, there was a significant accumulation of red cell choline as a result of lithium treatment. Since the increase in red cell choline (1-fold) was not as high as levels reported for patients maintained on lithium (8–10-fold), this suggests that species differences exist in red cell choline transport or metabolism.

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Endogenous biosynthesis of prostaglandin I_2 and thromboxane A_2 by isolated rat dental pulp

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Recent studies have revealed the heterogenous distribution of prostaglandin (PG) and thromboxane (TX) biosynthetic pathways in different tissues and cells. Dental pulp is a soft connective tissue encased in dentine and responsible for the formation and maintenance of dentine. PGE has been identified in homogenates of rat dental pulp [1]. Also PGE is released from isolated rat dental pulp into the incubation medium [1, 2], and from perfused tooth pulp of the dog by electrical stimulation of the dental nerves [3]. These releases of PGE are bloked by indomethacin, a cyclooxygenase inhibitor [4]. Although these findings suggest the presence of the cyclooxygenase pathway of arachidonic acid metabolism in the dental pulp, the PG and TX biosynthetic profile has been not determined. Attempts to measure biosynthetic capacity of pulp tissue homogenates by incubation with labelled precursor arachidonic acid showed no detectable conversion to PGs, probably due to exceedingly low capacity [1]. In the present study, therefore, the endogenous biosynthetic capacity of isolated dental pulp tissue to form PGE_2, $PGF_{2\alpha}$, PGI_2 and TXA_2 was investigated by radioimmunoassay.

Materials and methods

Materials. [^{3}H]PGE_2, [^{3}H]$PGF_{2\alpha}$ and [^{3}H]6-keto-$PGF_{1\alpha}$ were obtained from Radiochemical Center, Amersham, and [^{3}H]TXB_2 from New England Nuclear, Boston. PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, TXB_2 and anti-$PGF_{2\alpha}$ and anti-TXB_2 rabbit serum were gifts from Ono Pharmaceutical Co., Osaka. Anti-6-keto-$PGF_{1\alpha}$ rabbit serum was purchased from Seragen Inc., Boston, and anti-PGE_2 rabbit serum from Pasteur Institut, Paris. Indomethacin and tranylcypromine were obtained from Sigma Chemical Co., St. Louis, and thin layer silica gel plates (Kieselgel 60 F_{254}) from Merck, Darmstadt. Ionophore A23187 was a gift from Dr. R. Hamill, Eli Lilly and Co., Indianapolis, and OKY-046, (E)-[4-(1-imidazolylmethyl)phenyl]-2-propenoic acid hydrochloride, from Kissei Pharmaceutical Co., Nagano to Dr. Yusuo Endo.

Preparation and incubation of dental pulp. Adult male Wistar rats weighing 200–250 g were sacrificed by decapitation, and the mandibles and maxilla were excised. The dental pulp tissues were carefully removed in one piece from pulp cavities of incisors as described previously [2] and maintained in ice-cold physiological saline until use (30–60 min). In some expt., in order to remove blood the dental pulp was perfused *in situ* via carotid arteries with 4° Krebs–Henseleit bicarbonate buffer (pH 7.4) under pentobarbital anaesthesia. The isolated pulp tissues from one rat (approx 50 mg) were first preincubated for 30 min in 2 ml of Krebs buffer containing 1 mg/ml glucose, and then further incubated for 30 min in 2 ml of fresh Krebs buffer in the presence or the absence of test agents. When A23187 was used, dental pulp tissues were treated with A23187 during the preincubation period, and then incubated in fresh Krebs buffer not containing the ionophore. All incubations were carried out at 37° under a gas phase of 95% O_2 and 5% CO_2.

Extraction and assay of PGs and TXB_2. At the end of incubation, the medium was withdrawn, acidified with 0.5 M citric acid to pH 3.0–3.5, and extracted twice with 3 vol. of ethylacetate by collecting the organic phase by Pasteur pipette. The combined organic phase was evaporated to dryness under vacuum. Recoveries with this extraction procedure were constantly more than 90% as determined by expt. with tritium labelled PGs and TXB_2. Dried extract was redissolved in an appropriate volume of 0.1 M sodium phosphate buffer (pH 7.4) containing 0.9% (w/v) NaCl, 0.1% (w/v) NaN_3 and 0.1% (w/v) gelatin. Samples of 0.1 ml were subjected to radioimmunoassay (RIA) for PGE_2, $PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$ and TXB_2. These metabolites were also measured after further separation by TLC according to the method of Salmon (5). Briefly, an aliquot of