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The effect of narcotic analgesics on the uptake of 5-hydroxytryptamine and (−)-metaraminol by blood platelets

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Summary

1. The effects of narcotic analgesic and related drugs were studied on the uptake of 5-hydroxytryptamine (5-HT) and (−)-metaraminol by blood platelets.

2. The most potent drug in inhibiting the uptake of 5-HT (10 μM) by human platelets was methadone, followed by pentazocine>piminodine~pethidine~anileridine ~ cyclazocine ~ thebaine > dextropropoxyphene. Alphaprodine, papaverine, apomorphine, nalorphine, codeine, and morphine were almost without effect. Methadone was slightly less active than desipramine, and had 10% of the activity of imipramine under similar conditions. Naloxone did not antagonize the effect of methadone on 5-HT uptake.

3. The most potent inhibitor of metaraminol (3 μM) uptake by human platelets was piminodine, followed by pentazocine$\geqslant$anileridine>cyclazocine=methadone > dextropropoxyphene ~ thebaine $\geqslant$ papaverine ~ alphaprodine >pethidine>morphine. The activity of morphine was 1% of that of piminodine. Piminodine was more potent than desipramine and protriptyline under similar conditions. The order of potency of drugs studied in inhibiting the uptake of metaraminol by rabbit platelets was similar to that obtained with human platelets.

4. The effects of the analgesics studied on inhibiting uptake of monoamines did not correlate with their pain-relieving properties.

Introduction

The uptake and storage of 5-hydroxytryptamine (5-HT) by blood platelets closely resembles that which occurs in neurones. Moreover, the inhibition of this uptake by drugs is similar to that found in neurones (Paasonen, 1968 ; Paasonen, Ahtee & Solatunturi, 1971 ; Stacey, 1961 ; Todrick & Tait, 1969). We have recently shown that the orders of potency of tricyclic anti-depressants in inhibiting the uptake of 5-HT and a noradrenaline analogue, metaraminol, by human blood platelets were different. These drugs had a similar order of activity in inhibiting the uptake of 5-HT by platelets and in preventing the uptake of 5-HT by neurones, whereas their effects on the uptake of metaraminol by platelets paralleled their potencies in blocking the uptake of noradrenaline into neurones (Ahtee & Saarnivaara, 1971b).

The present work was undertaken to find out if narcotic analgesics inhibit the uptake of 5-HT and metaraminol by platelets because there is evidence that several

neurotransmitter amines are involved in the mechanism of the action of narcotic analgesics (Way & Shen, 1971). Some of the findings described in this paper were presented at the 24th Scandinavian Pharmacological Meeting (Ahtee & Saarnivaara, 1970) and the Third International Meeting of the International Society for Neurochemistry (Ahtee & Saarnivaara, 1971a).

Methods

Preparation of platelets

Buffy coats obtained from 400 ml of human citrated blood were diluted with an equal volume of modified calcium-free Tyrode solution (g/litre: disodium edetate 0·8, NaCl 7·6, KCl 0·42, $NaH_2PO_42H_2O$ 0·14, $NaHCO_3$ 2·1, glucose 2·0 and sucrose 4·5; pH 7·1–7·2) and platelets were separated by centrifugation at about 130 *g* for 20 min at 20° C. The final platelet suspension contained $8{\cdot}86 \pm 0{\cdot}41 \times 10^8$ platelets/ml (mean ± S.E.; 53 suspensions) and about one-tenth of the medium was original plasma. The pH of the final platelet suspension was 7·3–7·4. For the handling of the platelets only polypropylene vessels and pipettes were used.

Male albino rabbits weighing 2·5 to 3·5 kg were anaesthetized with ether and bled from the carotid artery by means of a polyethylene cannula. The blood was mixed with one-ninth volume of a 1·5% (w/v) solution of disodium edetate (EDTA) in 0·7% NaCl solution. The platelets were separated by centrifuging the blood at about 130 *g* for 20 min at 20° C. The platelet-rich plasma contained $5–10 \times 10^8$ platelets/ml.

In some experiments rabbit platelets were incubated in calcium-free Tyrode solution. The platelets were separated from plasma by centrifugation at about 2,500 *g* for 15 min at a temperature below 5° C. Thereafter the platelets were resuspended in a calcium-free Tyrode solution. The mean platelet count in these suspensions was about 3×10^8 platelets/ml.

Incubation of platelets

Release of 5-HT was studied by incubation, with gentle shaking of 4 ml duplicate samples of platelet suspension with or without drugs at 37° C, gassed with 96% O_2 and 4% CO_2. Drugs were added in a volume of 0·4 ml. Incubation was stopped by cooling the incubation tubes in an ice-bath. Thereafter the platelets were separated from the medium by centrifugation at about 2,500 *g* for 20 min below 5° C. The platelet-poor supernatant was decanted and traces of it remaining in the incubation tubes were wiped off with filter paper.

Uptake of 5-HT was studied by incubation of 2 ml duplicate samples of human platelet suspension under conditions described above with or without 5-HT (10 μM) or [^{3}H]-5-HT (G, 1 μM) of specific activity 50 mCi/mmol (Radiochemical Centre, Amersham). The drugs were added in a volume of 0·2 ml 10 min before 5-HT addition. Platelet pellets were prepared as described above.

To study the uptake of (−)-metaraminol bitartrate by rabbit platelets and the effect of metaraminol on the 5-HT content of platelets, 2 ml samples of rabbit platelet suspension were incubated under conditions described above for 1 hour. The final concentration of metaraminol ranged from 0·3 μM to 10 mM.

To study the effect of drugs on the uptake of metaraminol by rabbit platelets, 2 ml duplicate samples of platelet-rich plasma were incubated with the drugs for

15 min under the conditions described above and then metaraminol was added to give a final concentration of 3 μM ; the incubation was continued for 1 hour. Solutions of metaraminol and drugs or saline (0·9% w/v NaCl solution) were always added in a volume of 0·2 ml. After incubation the platelets were separated as described above. To study the effect of drugs on the uptake of metaraminol by human platelets, 2 ml duplicate samples of human platelet suspension were treated as the rabbit platelets except that the pre-incubation with drugs lasted for 10 min and that with metaraminol (3 μM) for 15 minutes.

Determination of 5-hydroxytryptamine and metaraminol

For the spectrophotofluorometric determination of 5-HT the platelet pellet was lysed with 1·5 ml of distilled water in a vortex mixer. The proteins were precipitated with a 10% (w/v) $ZnSO_4$ solution (0·2 ml) and 1 N NaOH (0·1 ml). The mixture was shaken well and centrifuged for 5 min at 700 *g*. After the addition of 12 N HCl (0·3 ml), the 5-HT was measured in 1 ml of the supernatant solution in an Aminco-Bowman spectrophotofluorometer (Weissbach, Waalkes & Udenfriend, 1958). All the drugs studied were checked for possible interference ; apomorphine disturbed the fluorometric assay of 5-HT and its effect on 5-HT uptake was therefore studied by using [^{3}H]-5-HT.

For liquid scintillation spectrometry the platelet pellets were solubilized by incubating them for 30 to 60 min at 60° C with 1 ml of a solution containing equal parts of Soluene 100 (Packard Instrument Co., Breda) and isopropranol and 0·2 ml of Perhydrol (E. Merck AG, Darmstadt). The clear solutions were added to 10 ml of scintillation fluid consisting of 0·4% (w/v) diphenyloxazole and 0·01% (w/v) 1,4-bis,2-(5-phenyloxazolyl-)-benzene in equal parts of toluene and ethylene glycol monoethylether. They were counted in a liquid scintillation counter Decem NTL 314 (Wallac Oy, Turku, Finland) after they had been allowed to stand for 24 h to eliminate autofluorescence. Quenching was corrected by the channel ratio method with an external standard.

The 5-HT content of platelets incubated with metaraminol was determined spectrophotofluorometrically by the method of Bogdanski, Pletscher, Brodie & Udenfriend (1956). The mean recovery of 5-HT with this method was 88·2 $\pm$ 2·2% (9 estimations).

The metaraminol content of platelets was determined after precipitating the proteins as described above. The metaraminol content of the supernatant was measured by the *o*-phtaldialdehyde method of Shore & Alpers (1964). In this procedure 101·9 $\pm$ 2·1% (12 estimations) of added metaraminol was recovered. In some of the experiments, metaraminol was extracted by the butanol-heptane procedure of Shore & Alpers (1964) ; the mean recovery of added metaraminol was 52·5 $\pm$ 3·2% (6 estimations). In all experiments in which the effect of drugs on the uptake of metaraminol was studied the $ZnSO_4$-procedure was used. In the concentrations used, only apomorphine interfered with the fluorescence of metaraminol.

Drugs

The drugs used were: ($\pm$)-alphaprodine hydrochloride (Hoffmann-La Roche Inc., Nutley), ($\pm$)-anileridine hydrochloride (Merck & Co. Inc., Rahway), (−)-apo-

morphine hydrochloride (Pharmacopoea Nordica), (−)-codeine phosphate (Orion Oy, Helsinki), (±)-cyclazocine base (Sterling-Winthrop Inc., New York), desipramine hydrochloride (Geigy, AG., Basle), dextropropoxyphene hydrochloride (Oy Astra Ab, Helsinki), 5-hydroxytryptamine creatinine sulphate (Fluka AG), imipramine hydrochloride (Geigy AG., Basle), (−)-metaraminol bitartrate (Merck, Sharp & Dohme, Philadelphia), (±)-methadone hydrochloride (Leiras Oy, Turku), (−)-morphine hydrochloride (Pharmacopoea Nordica), (−)-nalorphine bromide (Lääke Oy, Turku), (−)-naloxone hydrochloride (Endo Laboratories, Inc., Garden City), papaverine hydrochloride (Oy Medica Ab, Helsinki), (±)-pentazocine hydrochloride (Sterling-Winthrop Inc., New York), (±)-pethidine hydrochloride (Oy Star Ab, Tampere), (±)-piminodine ethanesulphonate (Sterling-Winthrop Inc., New York), (−)-thebaine hydrochloride (Verenigde Pharmaceutische Fabrieken N.V., Apeldoorn). Cyclazocine and piminodine were dissolved in a small amount of 0·1 N HCl, diluted with 0·9% w/v NaCl solution (saline) and neutralized with 0·1 N NaOH to pH 6·5–7·5. All the other drugs were dissolved in saline.

Results

Release of 5-hydroxytryptamine from platelets by analgesics

Table 1 shows the release of 5-HT from human blood platelets by narcotic analgesic and related drugs. None of the compounds studied caused a significant release of 5-HT at a concentration of 0·01 mM. At 0·1 mM thebaine, pentazocine, piminodine and methadone released 15 to 27% ; at 1 mM all of the compounds caused some reduction in platelet 5-HT content. Phenanthrene derivatives with the exception of thebaine were the weakest releasers. The most potent 5-HT releasers were methadone and its congener, dextropropoxyphene, followed by thebaine. At 1 mM pentazocine and its congener, cyclazocine, also released more than 60%. Piminodine could not be dissolved in a neutral solution of 1 mM.

TABLE 1. *Release of 5-hydroxytryptamine (5-HT) from human blood platelets by narcotic analgesics*

Drug	Mean (±S.E.) release at different drug concentrations (% of original content) 0·01 mM	0·1 mM	1 mM
Morphine	+3±1	3± 2	13± 2
Codeine		4± 1	21± 6
Nalorphine		7± 1	24± 3
Thebaine		15± 2	79± 2
Papaverine	1±2	10± 2	55±13
Pentazocine	5±2	22± 3	62± 4
Cyclazocine	10±1	10±10	61± 6
Pethidine	+1±3	6± 3	30± 2
Alphaprodine	6±5	5± 8	16± 3
Anileridine		11± 1	48± 3
Piminodine	+1±5	26± 6	
Methadone	5±3	27± 5	91± 2
Dextropropoxyphene		7± 1	87± 1

The mean values were calculated from 3–9 experiments, in which duplicate samples of platelet suspension were incubated for 1 h with or without drugs. Values marked with + indicate that the 5-HT content of drug treated platelets was higher than that of control platelets at the end of incubation. The original 5-HT content was 3·94±0·72 nmol/10^9 platelets.

Inhibition by analgesics of 5-hydroxytryptamine uptake by human platelets

Table 2 shows the inhibition by analgesics of 5-HT uptake by human blood platelets after 1 h incubation with 5-HT (10 μM). The mean 5-HT content in the control platelets increased by $245 \pm 12\%$ (69 experiments). Some experiments were carried out with [^{3}H]-5-HT (1 μM ; Table 3). The two procedures gave similar results. At 1 mM, morphine caused the smallest inhibition of 5-HT uptake and codeine, nalorphine and apomorphine were not very effective. Thebaine was the most potent of the morphine derivatives. Of the potent inhibitors, pentazocine, methadone, and anileridine showed an effect at the lowest concentration (0·01 mM).

TABLE 2. *Effect of narcotic analgesics on the uptake of 5-hydroxytryptamine (5-HT) by human blood platelets*

	Mean (±S.E.) inhibition at different drug concentrations (% of control uptake)		
Drug	0·01 mM	0·1 mM	1 mM
Morphine	7±3	3±3	29±8
Codeine	0±4	12±5	53±9
Nalorphine	0±4	18±6	34±8
Thebaine	0±3	40±9	93±1
Papaverine	1±1	7±3	68±8
Pentazocine	32±4	71±6	92±3
Pethidine	12±4	35±4	79±7
Anileridine	22±5	78±2	99±1
Methadone	30±5	85±4	82±1
Dextropropoxyphene	7±3	25±4	95±1

The drugs were added to the platelet suspension 15 min before 5-HT addition (10 μM); thereafter the incubation was continued for 1 hour. The mean values were calculated from 4–8 duplicate experiments. The % inhibition was obtained by dividing the difference between the net uptakes by control and drug-treated platelets by the net uptake by control platelets, multiplied by 100.

TABLE 3. *Effect of narcotic analgesics on the uptake of 5-hydroxytryptamine (5-HT) by human blood platelets*

	Mean (±S.E.) inhibition at different drug concentrations (% of control uptake)		
Drug	0·01 mM	0·1 mM	1 mM
Apomorphine	+8±2	18±10	79±7
Morphine	+10	1	36
Methadone	44	74	92

The platelets were incubated with [^{3}H]-5-HT (1 μM) instead of unlabelled 5-HT (10 μM). Otherwise the experiments were carried out as in Table 2. The mean values for apomorphine refer to 5 duplicate experiments, and those for morphine and methadone to one duplicate experiment. + Indicates that the uptake by drug-treated platelets was greater than that of control platelets.

The concentrations that cause 50% inhibition of uptake (IC50) indicate the order of potency of the analgesics (Table 4). During 15 min incubation with 5-HT the platelets accumulated 65% of the amount taken up in 1 h, i.e. the 5-HT content increased by $160 \pm 7\%$ (65 experiments). None of the drugs studied including imipramine, desipramine and protriptyline caused 5-HT release at the concentration at which they caused 50% inhibition of 5-HT uptake. The drugs caused inhibition of 5-HT uptake in concentrations which varied 100-fold, the slopes of the curves being nearly parallel with the exception of that obtained for methadone. The analgesic drug least active in these experiments was again morphine, the IC50 of which was 67 times greater than the IC50 of the most active drug, methadone.

However, because the methadone curve had a smaller slope than morphine these values are only approximate. The order of the inhibitory potency on the uptake of 5-HT by human platelets was methadone>pentazocine ($P<0.02$)>piminodine ($P<0.01$) ~ pethidine ~ anileridine ~ cyclazocine ~ thebaine > dextropropoxyphene ($P<0.05$)>alphaprodine ($P<0.01$)>morphine ($P<0.001$). Methadone was less active than imipramine ($P<0.02$), but more active than protriptyline ($P<0.05$).

To study the effect of a specific narcotic analgesic antagonist on the inhibitory action of narcotic analgesics on 5-HT uptake the human platelet suspension was incubated for 15 min with 5-HT (10 μM) after pre-incubation with naloxone (100 μM) for 15 min or with methadone (100 μM) for 10 min or with both naloxone (100 μM ; 15 min pre-incubation) and methadone (100 μM ; 10 min pre-incubation). Naloxone had no effect on the uptake of 5-HT, the 5-HT content of naloxone-treated platelets being $5 \pm 7\%$ (4 duplicate experiments) higher than that of the control platelets incubated with 5-HT only. Methadone alone inhibited the uptake of 5-HT by $58 \pm 7\%$ (3 duplicate experiments), and after both naloxone and methadone the uptake of 5-HT was inhibited by $62 \pm 9\%$ (3 duplicate experiments). At 1 mM, naloxone inhibited the 5-HT uptake by 30% (one duplicate experiment).

Inhibition by analgesics of metaraminol uptake by human platelets

Table 4 gives the IC50 values of the inhibitory effects of analgesics on (−)-metaraminol uptake. The human platelets were incubated with metaraminol (3 μM) for 15 min in the same manner as described for 5-HT. The drugs caused inhibition of metaraminol uptake in concentrations which varied 100-fold, the slopes of the curves being nearly parallel. The IC50 of morphine, the drug least active in these experiments, was nearly 100 times higher than that of the most potent inhibitor of metaraminol uptake, piminodine. The order of the inhibitory potency on the

TABLE 4. *Inhibition of the uptake of 5-hydroxytryptamine (5-HT) and (−)-metaraminol by human blood platelets by narcotic analgesic and antidepressant drugs*

Drug	IC50 for uptake of: Metaraminol (μM)	IC50 for uptake of: 5-HT (μM)	IC50 metaraminol/ IC50 5-HT	Approximate analgesic potency*
Morphine	1,570	1,360	1·2	1
Thebaine	208	123	1·7	0
Papaverine	290	450	0·6	0
Pentazocine	28	44	0·6	0·3
Cyclazocine	59	117	0·5	30
Pethidine	394	98	4·0	0·1
Alphaprodine	304	407	0·7	0·2
Anileridine	43	109	0·4	0·3
Piminodine	16	98	0·2	1·2
Methadone	60	21	2·9	1·2
Dextroproxyphene	186	207	0·9	0·04
Imipramine	963	3	337	
Desipramine	78	11	7·2	
Protriptyline	32	46	0·7	

The concentrations of the analgesic drugs which caused 50% inhibition of 5-HT and metaraminol uptake (IC50) were determined by calculating regression lines for the percentage inhibition of monoamine uptake against drug concentration (1 μM–30 mM). Drugs were added 10 min before the amines and the incubation was thereafter continued for 15 minutes. Four to seven duplicate experiments were performed with each drug and amine concentration. The IC50 of 5-HT uptake and metaraminol uptake by imipramine, desipramine and protriptyline obtained under identical conditions were taken from Ahtee & Saarnivaara (1971b). * Approximate analgesic potency in man (morphine=1) after subcutaneous injection (Goodman & Gilman, 1970; Hinshaw, Hobler, Borja & Sahler, 1966; Lasagna, De Kornfeld & Pearson, 1964).

uptake of metaraminol by human platelets was: piminodine$\geqslant$pentazocine$\geqslant$anileridine $>$ cyclazocine ($P<0{\cdot}05$)=methadone $>$ dextropropoxyphene ($P<0{\cdot}01$) $\sim$ thebaine$\geqslant$papaverine$\sim$alphaprodine$>$pethidine ($P<0{\cdot}05$)$>$morphine ($P<0{\cdot}001$). The effect of piminodine was different from that of anileridine ($P<0{\cdot}05$) and from that of the other analgesics ($P<0{\cdot}001$), with the exception of pentazocine. Piminodine was also more potent than protriptyline ($P<0{\cdot}001$), desipramine ($P<0{\cdot}001$), and imipramine ($P<0{\cdot}001$). The order of the inhibitory potency of the analgesics on metaraminol uptake was different from that of their inhibitory potency on 5-HT uptake. This is demonstrated by the ratios of IC50 of metaraminol uptake/IC50 of 5-HT uptake (Table 4).

TABLE 5. *Uptake of (−)-metaraminol by and release of 5-hydroxytryptamine (5-HT) from rabbit blood platelets incubated with various concentrations of metaraminol*

Metaraminol concentration (mM)	Mean uptake (±S.E.) of metaraminol (nmol/10^9 platelets)	Concentration gradient of metaraminol uptake (packed platelets/ incubation medium)	Mean (±S.E.) release of 5-HT (nmol/10^9 platelets)
0·0003	0·14± 0·07	47	0
0·001	0·37± 0·08	37	0
0·003	0·98± 0·15	33	0
0·01	2·9 ± 0·6	29	1·2±0·7
0·03	6·5 ± 1·5	22	9·1±2·9
0·1	19·4 ± 5·6	19	16·1±0·5
0·3	51·3 ± 19·7	17	25·2±4·1
1	139 ± 59	14	32·2±5·4
3	253 ± 96	8·4	43·0±3·6
10	559 ±317	5·6	50·0±3·2

2 ml samples of platelet suspension were incubated for 1 h at 37° C. Means (±S.E.) from 2–3 duplicate experiments. The mean uptakes are corrected for recovery of added metaraminol. The original 5-HT content was 55·9±1·4 nmol/10^9 platelets.

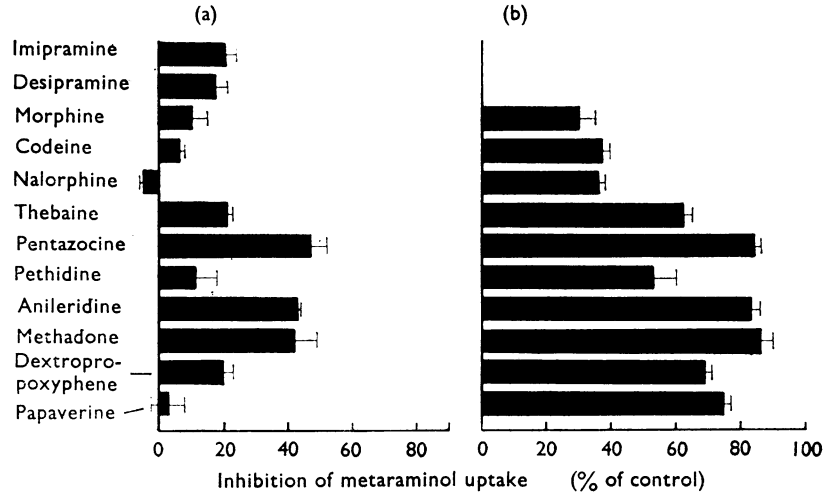

FIG. 1. Inhibition of the uptake of (−)-metaraminol by rabbit blood platelets by antidepressant and analgesic drugs. The drugs were added to 2 ml samples of platelet-rich plasma 15 min before addition of metaraminol (3 μM). The incubation was then continued for 1 hour. The horizontal columns indicate the mean inhibition (%) and the small bars represent the S.E. of the mean of 4–5 duplicate experiments. Abscissae, inhibition of metaraminol uptake expressed as % of control uptake. Ordinates, drugs used; concentrations, (a) 0·1 mM and (b) 1 mM.

Effect of analgesics on the uptake of metaraminol by rabbit platelets

Table 5 shows the uptake of (−)-metaraminol by rabbit platelets and the release of 5-HT as a function of extracellular metaraminol concentration. The platelets took up metaraminol against a concentration gradient which was inversely proportional to its concentration in the external medium. There was a certain stoichiometric relationship between the metaraminol taken up by platelets and the 5-HT released from them. The highest concentration of metaraminol used (10 mM) released almost all of the platelet 5-HT in 1 hour.

Figure 1 shows the inhibition by analgesics of the uptake of metaraminol (3 μM) by rabbit platelets. Three of the compounds, pentazocine, anileridine and methadone, were significantly more potent as inhibitors than imipramine or desipramine ($P<0{\cdot}01$–$0{\cdot}001$) inhibiting by 40% or more at 0·1 mM; they were ineffective at 0·01 mM.

Discussion

Our results show than certain narcotic analgesics such as pentazocine, pethidine, methadone and their derivatives inhibit the uptake of 5-HT by blood platelets *in vitro*. However, as found by Stacey (1961), morphine was practically without effect on uptake. Methadone, the most effective drug in these experiments, was about half as active as desipramine and one-tenth as active as imipramine under similar conditions (Ahtee & Saarnivaara, 1971b). Of the analgesics, pentazocine and piminodine were found to be more potent inhibitors of metaraminol uptake than desipramine and protriptyline which are two of the most potent inhibitors of noradrenaline uptake by nerve endings (Callingham, 1967; Carlsson, Corrodi, Fuxe & Hökfelt, 1969b). The weakest inhibitor of metaraminol uptake by both human and rabbit platelets was morphine, followed by other phenanthrene derivatives.

The analgesics studied had approximately similar orders of potency in inhibiting the metaraminol uptake by human and rabbit platelets. However, 2 to 3-fold higher concentrations were needed to produce a degree of inhibition of the uptake by rabbit platelets similar to that obtained with human platelets. This apparent discrepancy is probably explained by the longer incubation period and by the probable binding of drugs to plasma proteins in the rabbit platelet-rich plasma. The incubation conditions have a considerable effect on the absolute concentrations of drugs needed to inhibit monoamine uptake; thus Tuomisto (1972) found that, when he incubated with 5-HT 0·1 μM for 1 min, 0·1 μM of imipramine inhibited 50% of the 5-HT uptake by rabbit platelets whereas a 30 times higher concentration of imipramine was needed to cause a similar inhibition of 5-HT uptake by human platelets under the incubation conditions used by us (Ahtee & Saarnivaara, 1971b) or by Todrick & Tait (1969).

The order of potency of the narcotic analgesics in inhibiting the uptakes of metaraminol and 5-HT by platelets were dissimilar. The potencies of tricyclic antidepressants in inhibiting the uptake of 5-HT and metaraminol by platelets differ in the same way as their potencies in inhibiting the uptake of 5-HT and noradrenaline by neurones (Ahtee & Saarnivaara, 1971b). Therefore, the narcotic analgesics could act on either the neuronal 5-HT or noradrenaline uptake. In fact, Carlsson & Lindqvist (1969) showed by a displacement technique that methadone

and pethidine prevent the monoamine uptake by 5-HT neurones in lower concentrations than they do the uptake into noradrenaline neurones. The observations that an antidepressant drug may inhibit the uptake of one monoamine more than that of another, has been thought to be associated with the existence of different transport mechanisms for different monoamines, the sensitivities of different transport systems towards different drugs being dissimilar (Iversen, 1971 ; Snyder, Kuhar, Green, Coyle & Shaskan, 1970). However, the dissimilarities in the order of potency of narcotic analgesics and tricyclic antidepressants in inhibiting the uptake of 5-HT and metaraminol by blood platelets, suggest that in addition to a specific transport mechanism the molecular structure of the monoamine accumulated is of importance in determining the inhibitory potency of a drug. Another explanation for our results is, of course, that platelets have different uptake systems for different amines.

Certain relationships between the structures of narcotic analgesics and their effects suggest that similar structural features to those of tricyclic antidepressants could be involved in the effects of narcotic analgesics on the monoamine transport. The most potent narcotic analgesic to inhibit 5-HT uptake, methadone, has a tertiary amine group in the dimethylaminopropyl side-chain. This agrees with the well-established fact that the tricyclic antidepressants with a tertiary amine group are the most potent inhibitors of 5-HT uptake both by neurones and platelets (Ahtee, Tuomisto, Solatunturi & Paasonen, 1968 ; Carlsson, Corrodi, Fuxe & Hökfelt, 1969a, b ; Todrick & Tait, 1969). The high activity of piminodine in inhibiting the uptake of metaraminol could be related to the secondary amine in the alkylamino side-chain. The secondary amine structure is also a prominent feature of those antidepressant drugs which are the best inhibitors of noradrenaline uptake into neurones (Callingham, 1967 ; Carlsson *et al.*, 1969a, b). Moreover, the secondary amine group in the piminodine molecule is probably involved in its comparatively high potency in releasing 5-HT because the most potent releasers of 5-HT among phenothiazine derivatives and tricyclic antidepressants are the compounds with a secondary amine group (Ahtee, 1966 ; Ahtee & Paasonen, 1968 ; Ahtee *et al.*, 1968).

The inhibitory effects of the narcotic analgesics on 5-HT and metaraminol uptake were not correlated with their analgesic potencies (Table 4). The specific antagonist, naloxone, did not antagonize the inhibitory effect of methadone on 5-HT uptake. Therefore it is plausible that the part of the molecule which determines the affinity of these analgesics for narcotic receptors is not involved in their ability to inhibit the uptake of 5-HT or metaraminol by platelets. However, there is evidence that 5-HT, noradrenaline and dopamine modify the analgesic action of morphine and related drugs (Saarnivaara, 1969a, b ; Takagi & Nakama, 1968 ; Tenen, 1968). Moreover, the inhibitory effect of analgesics on monamine uptake could at least partially explain the toxic reactions seen when analgesics are combined with drugs which increase the monoamine concentration at synapses (Loveless & Maxwell, 1965 ; Mustala & Jounela, 1966). Therefore, although the inhibitory effects of narcotic analgesics on monoamine uptake by platelets do not correlate with their pain-relieving properties, the possibility cannot be excluded that the inhibition of monoamine uptake may modify the analgesic and other effects of narcotic analgesics.

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