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Alkylxanthines as research tools[☆]

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Abstract

(1) The methylxanthine caffeine has many pharmacological effects, most of which can be linked to blockade of adenosine receptors, inhibition of phosphodiesterases, and augmentation of calcium-dependent release of calcium from intracellular stores. (2) A variety of xanthines have been developed as potent and/or selective antagonists for adenosine receptors. (3) Several xanthines have been developed that are more potent and more selective inhibitors of cyclic nucleotide phosphodiesterase than caffeine or theophylline. (4) Caffeine remains the xanthine of choice for activation of intracellular calcium-sensitive calcium release channels although millimolar concentrations are required, which can have effects on other aspects of calcium regulation.

Keywords: Caffeine; Methylxanthines; Pharmacological effects

1. Introduction

The natural methylxanthines, caffeine and theophylline, have marked effects on the cardiovascular, respiratory, renal, and nervous systems. During the past century, there has been extensive research into the mechanisms involved in such actions of caffeine (Daly, 1993, 1998; Fredholm et al., 1997). Four sites appear most relevant to the in vivo pharmacological and toxicological effects of caffeine; these are (a) the blockade of adenosine receptors, (b) the inhibition of cyclic nucleotide phosphodiesterases, (c) the sensitization to calcium of the cyclic ADP ribose-modulated calcium-release channel associated with certain intracellular stores of calcium, and (d) the inhibition of $GABA_A$ receptors. Many xanthine analogs have been synthesized in hopes to obtain more selective and potent agents for use both as research tools and as possible therapeutic agents. Some potential uses are as antiasthmatics, analgetic adjuvants, antitussives, bronchodilators, cardiac stimulants, cognition enhancers, diuretics, lipolytic agents, and cancer chemotherapy adjuvants, and for treatment of cerebral ischemia, Parkinson's disease and renal failure. Some representative xanthines useful as research tools are shown in Fig. 1.

2. Adenosine receptors

The antagonism by caffeine of the effects of adenosine on heart was reported by DeGubareff and Sleator (1965), providing evidence for what would later prove to be xanthine-sensitive adenosine receptors in heart and other tissues. In 1971, specific antagonism by methylxanthines of adenosine-elicited accumulation of cyclic AMP in brain slices was reported (Sattin and Rall, 1970). Burnstock, in a remarkable synthesis derived from the literature on ATP- and adenosine-mediated responses, correctly formulated two classes of purinergic receptors, namely the P_1-receptors at which adenosine was much more potent than ATP and which were effectively blocked by methylxanthines, and the P_2-receptors at which ATP and/or other nucleotides were active and which were insensitive to methylxanthines (Burnstock, 1978). The interrelationship of ATP as a purinergic agent and adenosine as a biologically active metabolite was clearly formulated by Burnstock, and methylxanthines, as selective blockers of any adenosine component to the ATP responses, became important research tools.

The P_1-receptors were subsequently divided into two subclasses, the A_1- and A_2-adenosine receptors, the former being inhibitory to adenylyl cyclase and the latter stimulatory to adenylate cyclase (Londos and Wolff, 1977; Van Calker et al., 1979). A further division of the A_2-receptors

[☆]This overview is dedicated to Geoffrey Burnstock, who continues to be a pioneer in research on the function of purinergic systems.

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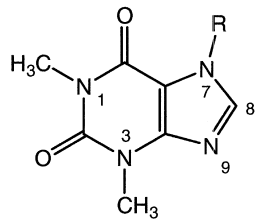
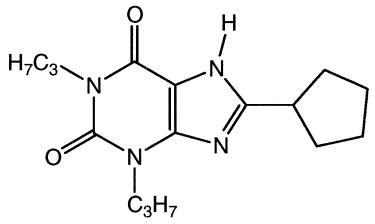


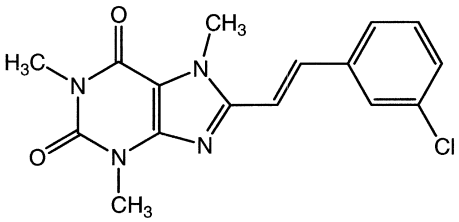
Caffeine R = CH_3
Theophylline R = H

P_1-Antagonist
PDE Inhibitor
Calcium Releaser



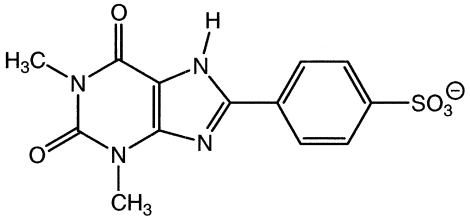
CPX

A_1-Antagonist



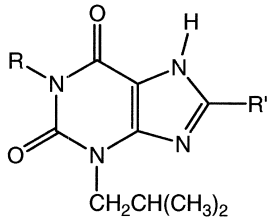
CSC

A_{2A}-Antagonist



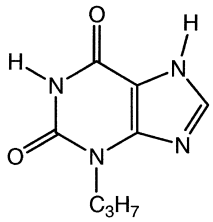
8-pSPT

Anionic P_1-Antagonist



IBMX R = CH_3, R' = H Non Selective PDE Inhibitor, P_1-Antagonist

IIX R = $(CH_2)_2CH(CH_3)_2$, R' = H Class IV PDE Inhibitor



Enprofylline

A_{2B}-Antagonist

8-MeOCH$_2$-IBMX R = CH_3, R' = CH_2OCH_3 Class I PDE Inhibitor

Fig. 1. Representative xanthines useful as research tools.

into the high agonist affinity A_{2A}-receptors and low agonist affinity A_{2B}-receptors followed (Daly et al., 1983; Bruns et al., 1986). The existence of subtypes of adenosine receptors, subserving different physiological functions, provided the impetus for the development of selective and potent xanthine antagonists for adenosine receptors. A third major class of adenosine receptors, the A_3-receptor has now been recognized. Xanthines did not prove to be satisfactory as selective antagonists for A_3-receptors, having low affinity for rat A_3-receptors (Kim et al., 1994), but somewhat higher affinity for human A_3 receptors (Salvatore et al., 1993).

The discovery that introduction of an 8-phenyl moiety into theophylline markedly enhanced antagonist activity at A_{2B} receptors activating cyclic AMP accumulation (Bruns, 1981) provided a key direction for further structural modification. A combination of larger substituents at the 1 and 3 position with the 8-phenyl substituent led to 1,3-dipropyl-8-phenylxanthine, a potent and selective antagonist for A_1 receptors (Bruns et al., 1980). The 8-phenyl xanthines had the disadvantage of low aqueous solubility and high lipophilicity. 8-*p*-Sulfophenyltheophylline (8-pSPT) and 1,3-dipropyl-8-*p*-sulfophenylxanthine were introduced as water-soluble antagonists (Bruns et al., 1980; Daly et al., 1985) that would not penetrate into cells and hence would not affect phosphodiesterases or calcium-release channels; nor would such anionic xanthines penetrate into brain. A ‘functionalized congener’ approach provided XAC, a 1,3-dipropyl-8-phenylxanthine with a *p*-$OCH_2CONH(CH_2)_2NH_2$ substituent, as a potent, mod-

erately A_1-selective antagonist with increased water solubility (Jacobson et al., 1986). The enhancing effect of 8-cycloalkyl substituents on activity, first reported by Bruns (1981), was the starting point for the development of 8-cyclopentyl-1,3-dipropylxanthine (CPX), a very potent and very selective A_1-adenosine receptor antagonist (Bruns et al., 1987; Shamim et al., 1988). An apparent stimulation of adenylyl cyclase in rat adipocyte membranes by 3-isobutyl-1-methylxanthine (IBMX) (Parsons et al., 1988) is most likely due to binding as an antagonist to the A_1-adenosine receptor in such membranes thereby preventing agonist-independent inhibition of the cyclase through the receptor-G_i complex.

The development of truly A_{2A}-selective xanthines began with observation of modest A_2-selectivity of 8-cyclohexylcaffeine (Shamim et al., 1989), which led to development of 8-styryl-1,3,7-trisubstituted xanthines, such as 8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-methylxanthine (KF-17837) (Nonaka et al., 1994), 8-(3-chlorostyryl)caffeine (Jacobson et al., 1993) and 8-(3-chlorostyryl)-3,7-dimethyl-1-propargylxanthine (Müller et al., 1997). Such 8-styrylxanthines are potent and highly A_{2A}-selective. 3,7-Dimethyl-1-propargylxanthine (DMPX) has been used as an A_2-selective antagonist, but the in vitro selectivity is only about fourfold (Seale et al., 1988; Daly et al., 1991).

There is current interest in xanthines with selectivity as antagonists for A_{2B} receptors because of possible antiasthmatic activity. The antiasthmatic agent 3-propylxanthine (enprofylline) is a relatively weak antagonist at A_1 and A_{2A} receptors and has proved to be a somewhat selective but not potent A_{2B} antagonist (Robeva et al., 1996; Linden et al., 1999). A 1,2-dimethylmaleylhydrazide of xanthine carboxylic acid (XCC, 8(4-carboxymethyloxyphenyl)-1,3-dipropylxanthine) is a potent and selective antagonist for human A_{2B} receptors (Kim et al., 1999).

Further developments in xanthine antagonists selective for A_1-, A_{2A}- and A_{2B}-adenosine receptors are covered in recent reviews (Baraldi et al., 1995; Feoktistov and Biaggioni, 1997; Müller, 1997). Several xanthines have been developed as radioligands for adenosine receptors.

Xanthines can also affect physiological functions of adenosine by blocking adenosine uptake mechanisms. The inhibition of adenosine uptake by xanthine derivatives, including propentofylline (7-(5-oxohexyl)-3-methyl-7-propylxanthine) (Fredholm et al., 1994; Parkinson et al., 1994), pentoxifylline (7-(5-oxohexyl)theobromine) (Fredholm et al., 1994) and denbufylline (7-(2-oxopropyl)-1,3-dibutylxanthine) (Nicholson et al., 1989) may contribute to antiischemic activity. Such xanthines also inhibit phosphodiesterases and have antiasthmatic activity.

3. Phosphodiesterases

The first use of caffeine as a cyclic nucleotide phosphodiesterase (PDE) inhibitor was during the discovery of cyclic AMP and of the enzymes responsible for cyclic AMP formation and inactivation (Rall and Sutherland, 1958; Sutherland and Rall, 1958). A 6.7 mM concentration of caffeine was used to inhibit PDE. Potentiation of a hormone response by a methylxanthine was to become one criteria for involvement of a cyclic nucleotide in that response. Intensive research programs aimed at developing xanthines more potent than caffeine/theophylline as phosphodiesterase inhibitors were initiated, since such xanthines could have potential as antiinflammatories, bronchodilators, diuretics, cardiac stimulants, vasodilators and lipolytics. A series of 56 xanthines were assessed as PDE inhibitors and lipolytic agents (Beavo et al., 1970). 3-Isobutyl-1-methylxanthine (IBMX) proved to be 15-fold more potent than theophylline in both assays. Later it was realized that lipolytic activity in fat cells related more to blockade of A_1-adenosine receptors inhibitory to adenylyl cyclase than to inhibition of PDE and IBMX did prove to be more potent than theophylline as an antagonist at adenosine receptors. The last three decades saw scores of reports on new xanthines as phosphodiesterase inhibitors along with correlations with myotonic, cardiotonic, bronchodilator and antiasthmatic effects. There are many PDE isozymes with five major classes being proposed in 1990 (Beavo and Reifsnyder, 1990). Caffeine and theophylline appear non-selective and relatively weak as PDE inhibitors with IC_{50} values usually greater than 500 μM for caffeine and usually greater than 100 μM for theophylline. Although more potent with IC_{50} values of about 10 μM, IBMX also is relatively non-selective with respect to the five classes of PDE isozymes (Ukena et al., 1993). IBMX came to be used as a prototypic 'nonselective' PDE inhibitor. Since the PDE isozymes are expressed to differing degrees in different cells and tissues and, thus, subserve different functions, the development of xanthines with selectivity towards PDE subtypes was important. The pioneering research of Wells et al. (1981) led to some xanthines with selectivity towards either the class I PDE or the class IV PDE. 3-Isobutyl-1-isoamylxanthine (IIX) was about fourfold more potent (IC_{50} 17 μM) at the class IV PDE. Certain 8-substituted xanthines such as 8-methoxymethyl-IBMX (IC_{50} 5 μM), were 40-fold more potent for the class I PDE. IIX is relatively weak as an adenosine receptor antagonist and potentiated adenosine-mediated accumulations of cyclic AMP in brain slices (Smellie et al., 1979a,b). Thus, IIX appears to represent a somewhat selective probe for the class IV PDE with little activity at adenosine receptors. 8-Methoxymethyl-IBMX, selective for the class I PDE, does not seem to have been assessed as an adenosine antagonist. It would be predicted to be a weak antagonist.

A comparison of 11 xanthines, including caffeine, theophylline, IBMX, enprofylline and 8-phenyltheophylline as inhibitors of the five classes of PDE isozymes and as antagonists of A_1 and A_2-adenosine receptors has appeared (Ukena et al., 1993). Xanthines with high

potency as inhibitors of a brain class IV PDE appear to be behavioral depressants even though many are more potent as adenosine receptor antagonists than the behavioral stimulants caffeine and theophylline (Snyder et al., 1981; Choi et al., 1988).

4. Calcium

The stimulatory effects of caffeine on skeletal, cardiac and smooth muscle had been known for several decades prior to the discovery that caffeine-elicited contractures were due mainly to increases in intracellular calcium (Bianchi, 1961; Frank, 1962). The putative target for the effect of caffeine is a cyclic ADP ribose-modulated, ryanodine-sensitive calcium-release channel at which caffeine increases the sensitivity of the channel to activation by calcium (McPherson et al., 1991). Ryanodine at low concentrations has the same effect as caffeine, causing opening of the channel, while at higher micromolar concentrations ryanodine causes blockade of the channel. Caffeine usually requires 1 mM or higher concentrations to elicit marked release of intracellular calcium. However, sodium-dependent calcium-release from rat heart sarcolemmal preparations was markedly enhanced by 100 μM caffeine (Gupta et al., 1990). Caffeine has become established and widely used at the requisite millimolar concentrations to study the function of calcium-sensitive ryanodine receptor-channels, but it is now apparent that caffeine has other effects on the complex mechanisms involved in regulating calcium levels in intact cells. Thus, caffeine in *Xenopus* oocytes inhibited inositol trisphosphate (IP_3)-mediated release of intracellular calcium from IP_3-sensitive pools (Parker and Ivorra, 1991). At 1 mM concentrations caffeine had a marked effect, while theophylline had only a slight effect and IBMX had none. Caffeine also inhibited IP_3-mediated release of calcium in permeabilized rat hepatocytes (Missiaen et al., 1992) and permeabilized guinea pig smooth muscle (Hirose et al., 1993) and in cerebellar microsomal preparations (Brown et al., 1992; Ehrlich et al., 1994). In histamine-stimulated HeLa cells, caffeine and theophylline inhibited calcium oscillations and both caffeine and theophylline at 10 mM or greater blocked histamine-elicited IP_3-formation (Diarra et al., 1994). Neither alone caused release of intracellular calcium. Others have reported inhibition of agonist-induced calcium oscillations in hepatocytes (Combettes et al., 1994; Sanchez-Bueno et al., 1994). Caffeine blocked calcium signals elicited by acetylcholine, cholecystokinin and ATP in pancreatic acinar cells apparently by inhibiting IP_3 formation (Toescu et al., 1992). In salivary acinar cells, caffeine inhibited calcium signals and IP_3-formation by acetylcholine and norepinephrine, but not those elicited by substance P (Seo et al., 1999). A variety of effects of millimolar concentrations of caffeine on calcium clearly appear possible, including enhanced calcium-induced calcium release through ryanodine-sensitive channels, inhibition of IP_3-induced calcium release, blockade of receptor-mediated IP_3 formation, and enhanced reuptake of calcium into IP_3-sensitive pools. Thus, caffeine needs to be used cautiously as a research tool for the study of calcium regulation in intact cells.

There have been few structure–activity studies with respect to xanthines and calcium-invoked calcium release through ryanodine-sensitive channels. With sarcoplasmic reticular vesicles incorporated into lipid bilayers, efflux rates for radioactive calcium were increased by 1.25 mM concentrations of caffeine, theophylline, paraxanthine, theobromine, IBMX and 3,9-dimethylxanthine (Rousseau et al., 1988). In pheochromocytoma cells, a series of 38 xanthines were assessed for effects on intracellular calcium (Müller and Daly, 1993). At 10 mM concentrations several xanthines elicited a two- to threefold greater elevation of calcium than did caffeine. These were 1-propyltheobromine, 1-propargyltheobromine, and 3-propargylparaxanthine. The 1-propyl and 1-propargyl analogs were about four times more potent than caffeine with EC_{50} values of about 5 mM. IBMX at 1 mM, which is about the limit of water-solubility, had no effect. In contrast, in dorsal root ganglion neurons IBMX at 5 mM was reported to elicit an increase in intracellular calcium similar to that elicited by 10 mM caffeine and theophylline (Usachev and Verkhratsky, 1995). Recently, a series of 39 xanthines were assessed for effects on calcium levels in preparations of sea urchin eggs (Cavallaro et al., 1999). At 1 mM concentrations, most were less effective than caffeine, but 7-ethylcarboxypentyltheophylline and 7-(6′-hydroxyheptyl)theophylline were slightly more potent than caffeine. The former was also slightly more efficacious. 1-Propargyl-7-(6′-oxoheptyl)-3-methylxanthine was fourfold more potent than caffeine.

Caffeine can augment the calcium-dependent binding of [^{3}H]ryanodine to the channel by potentiating the stimulatory effect of calcium ions (Pessah et al., 1987). A series of 30 xanthines were assessed for effects on [^{3}H]ryanodine to striated muscle sarcoplasmic reticulum in the presence of suboptimal concentrations of calcium (Liu and Meissner, 1997). At 1.5 mM concentrations, theophylline, theobromine, and IBMX were nearly as efficacious as caffeine, while 1-propargyltheobromine, 1,3-dipropyl-7-methylxanthine, paraxanthine, 7-β-chloroethyltheophylline, 7-methylxanthine, 7-propylxanthine and 3-propylxanthine were slightly more efficacious than caffeine. The most effective xanthine was 1-hexyltheobromine.

Further studies may lead to xanthines with greater specificity for ryanodine-sensitive channels, but the results to date suggest that the interaction is of very low affinity and no structural leads towards greatly enhanced affinity have been forthcoming. Thus, caffeine remains a useful but far from satisfactory research tool for studying calcium release.

Caffeine has been reported to elicit influx of calcium

through a plasma membrane channel in smooth muscle cells (Guerrero et al., 1994), in a PC12-37 pheochromocytoma clone lacking the ryanodine-sensitive channel (Avidor et al., 1994) and in a smooth muscle DDT_1-MF-2 cell treated with thapsigargin to arrest cell growth (Ufret-Vincenty et al., 1995). In addition to such novel activation of calcium influx, caffeine at 10 mM has been reported to suppress L-type calcium currents (Kramer et al., 1994; Yoshino et al., 1996).

5. $GABA_A$ receptors

Caffeine and theophylline inhibit binding of GABA and diazepam to $GABA_A$ receptors in brain membranes (Marangos et al., 1979; Ticku and Burch, 1980). However, the affinities are very low (K_I>300 μM). IBMX is somewhat more potent than theophylline or caffeine. Certain water-soluble pyrimidotheophyllines are relatively potent diazepam antagonists with K_I values of 20–30 μM, but such pyrimidotheophyllines also have affinities for A_1 and A_2 receptors of 20–30 μM (Geis et al., 1995). Further structure–activity relationships for xanthines at $GABA_A$ receptors have not been forthcoming. Caffeine at a concentration of 50 μM inhibited muscimol-elicited chloride flux in brain synaptoneurosomes (Lopez et al., 1989). Such functional inhibitions of $GABA_A$ receptors may involve caffeine-induced calcium release, resulting in calcium-mediated inhibition of $GABA_A$ receptor-mediated responses (Desaulles et al., 1991; Kardos and Blandl, 1994). However, in rat hippocampal neurons inhibition of GABA- and glycine-elicited chloride currents by caffeine did not appear to involve elevation of intracellular calcium (Uneyama et al., 1993). Caffeine inhibited the glycine response with an IC_{50} of 500 μM, while the IC_{50} for the GABA response was about 4 mM. Pentoxifylline was somewhat more potent than caffeine in inhibiting the glycine response; theophylline was equipotent, paraxanthine somewhat less potent, and theobromine and IBMX threefold less potent.

6. Ion channels

Caffeine at millimolar concentrations can inhibit various ion channels, including L-type calcium channels (see Section 4), voltage-sensitive sodium channels (Habuchi et al., 1991) and voltage-sensitive potassium channels (Reiser et al., 1996). IBMX can also inhibit potassium channels (Usachev et al., 1995; Reiser et al., 1996) and appears to be a channel blocker at neuromuscular nicotinic receptors (Akasu and Karczmar, 1980). Apparent stimulatory effects of caffeine and IBMX on calcium-dependent potassium channels (Usachev et al., 1995) probably relate to xanthine-elicited increases in intracellular calcium. Other effects of caffeine on ion channels may also relate to calcium release.

The effects of xanthines on chloride efflux through a cyclic AMP-activated channel have been studied by several groups during the past decade, particularly with regard to the cystic fibrosis membrane regulator (CFTR) in which a mutation reduces sensitivity of the channel to cyclic AMP and confers a sensitivity to activation by certain xanthines (Eidelman et al., 1992). The most effective xanthine was 8-cyclopentyl-1,3-dipropylxanthine with other xanthines being less active or inactive (Guay-Broder et al., 1995; Jacobson et al., 1995). A forskolin-mediated stimulation of chloride flux was augmented by relatively high concentrations of 8-cyclopentyl-1,3-dipropylxanthine (EC_{50} 60 μM) and IBMX (EC_{50} 1.5 mM) (Haws et al., 1996). The lack of effect of 8-cyclopentyl-1,3-dipropylxanthine on conductances in cells with the mutant CFTR was interpreted as indicating that there was no direct effect of the xanthine on the CFTR-modulated chloride conductance (Kunzelmann et al., 1998). A recent study with CHO cells transfected with wild type CFTR reported the effects of 35 xanthines (Chappe et al., 1998). A wide range of xanthines including caffeine, theophylline, IBMX, enprofylline, 1-isobutyltheobromine and 1,3-dipropyl-7-methylxanthine were effective stimulants at 500 μM. IBMX was the most efficacious. 8-Substituted xanthines, including 8-cyclopentyl-1,3-dipropylxanthine, were ineffective.

7. Enzymes

Caffeine and theophylline at millimolar concentrations have been reported to inhibit various enzymes including phosphorylase a (Ercan-Fang and Nuttall, 1997), guanylate cyclase (Strinden and Stellwagen, 1984), Na^+–K^+-ATPase (Gupta et al., 1990) and 5′-nucleotidases (Fredholm et al., 1978; Fredholm and Lindgren, 1983). IBMX at 1 mM inhibited phospholipase-catalyzed arachidonate release and cyclooxygenase-catalyzed oxidation of arachidonate (Whorton et al., 1985). Furafylline (1,8-dimethyl-3-(2′-furfuryl)methylxanthine) is a potent inhibitor of cytochrome P450 (Sesardic et al., 1990).

8. Other effects

Caffeine, theophylline, IBMX, pentoxifylline and other xanthines can inhibit cell proliferation (Levi-Schaffer and Touitou, 1991; Windmeier and Gressner, 1996) presumably through inhibition of PDE, resulting in elevation of cyclic AMP. Caffeine and pentoxifylline at 100–300 μM inhibited antigen-elicited proliferation of lymphocytes

(Rosenthal et al., 1992). Similarly, a variety of xanthines blocked antigen-induced activation of a tumor mast cell line, apparently by inhibition of antigen-binding to an immunoglobulin E (Ali et al., 1991).

The antagonism by caffeine of mitotic arrest in the G_2 checkpoint suggests that caffeine or a less toxic xanthine analog would be useful as an adjuvant for cancer chemotherapy (Musk and Steel, 1990; Rowley et al., 1984). Cancer cells that lack P53 cannot utilize the G_1 checkpoint and, thus, only the G_2 checkpoint is available for DNA repair. Pentoxifylline has the same effect as caffeine (Fan et al., 1995; Russell et al., 1996). The basis for the ability of xanthines, such as caffeine and pentoxifylline, to prevent G_2 arrest is unknown. It has been proposed that an activation by caffeine of a P34 cdcz protein kinase is involved (Yao et al., 1996). Recently, inhibition of ATM and ATR protein kinases by caffeine has been proposed as a key mechanism for prevention of arrest in G_2 phase (Sarkaria et al., 1999). Fifty-five xanthines have been assessed for effects on P53-defective tumor cells relative to prevention of G_2 arrest and thus DNA repair (Jiang et al., 2000). Caffeine and nine other xanthines showed activity with IC_{50} values less than 2 mM. The most active were the 1-ethyl, 1-propyl- and 1-hexanonyl- (pentoxifylline) analogs of caffeine.

9. Conclusion

Caffeine and theophylline as prototypic xanthines continue to enjoy widespread use as research tools and have spawned many generations of structurally modified xanthines with greater potency and/or selectivity towards the many biological targets with which caffeine and theophylline interact. The initial proposal by Burnstock (1978) that blockade by methylxanthines was one criterion for involvement of P_1 receptors in a response has now seen the development of many xanthines useful for characterizing the subclasses of P_1 receptors and their role in physiological functions of adenosine. The inhibition of phosphodiesterases by caffeine and theophylline led to the development of much more potent and in some cases more selective xanthines, such as IBMX, IIX, and 8-methoxymethyl-IBMX, for study of this enzyme. A wide range of xanthines with potential as bronchodilators have been prepared, many of which have a range of biological targets, including adenosine receptors, adenosine transporters and phosphodiesterases. As yet no xanthines with much greater potency than caffeine have been developed as tools for investigating calcium-activated calcium release channels. Other targets for millimolar concentrations of caffeine also remain elusive with respect to discovery of xanthines with greater potency or selectivity. Thus, there remain many challenges for further development of xanthines as potent and selective research tools.

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